Patent Docket P1101P2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	Group Art Unit: 1647
Avi J. Ashkenazi, et al.	Examiner: Kaufman, C.
Serial No.: 09/396,710	**Customer No:09157**
Filed: September 15, 1999	
For: Apo-2 Receptor Antibodies	

RULE 131 DECLARATION

I, Avi J. Ashkenazi, hereby declare as follows:

1. I am the named inventor of the claimed subject matter of the above-identified patent application.

2. The above-identified patent application claims priority to application serial no. 08/857,216 filed with the Patent Office on May 15, 1997, and I am the named inventor in that priority application. A copy of my priority application serial no. 08/857,216 (hereinafter the "'216 application") is attached as Exhibit A.

3. All work described in the above-identified application and the '216 application was performed by me or on my behalf in the United States of America.

4. The '216 application filed on my behalf on May 15, 1997 demonstrates both my conception of the claimed invention of the present application and a constructive reduction to practice of the invention.

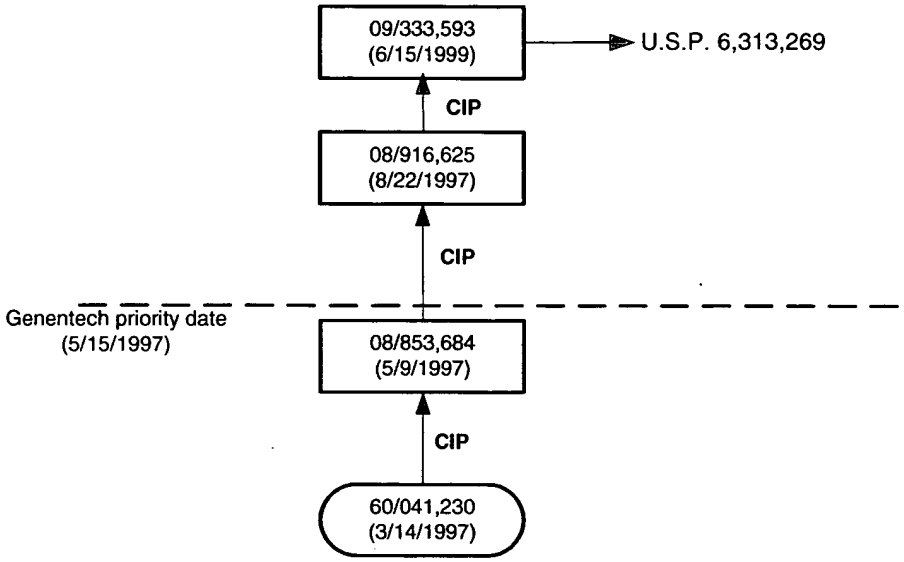
5. Experiments performed by me or on my behalf relating to the identification and structural characterization of the Apo-2 receptor are described, for example, in Example 1 of the '216 application, pages 58-62. In *in vitro* binding assays, I found that the Apo-2 receptor extracellular domain binds the ligand known as Apo-2 ligand (the '216 application, e.g., pages 63, lines 9-35 - page 64, lines 1-6). In further *in vitro* assays, I also found that the Apo-2 receptor was capable of inducing cell death in transfected mammalian cells (the '216 application, page 64, lines 9-35 - page 65, lines 1 -13).

6. In the '216 application, agonist antibodies to the Apo-2 receptor are described. (See, e.g., Page 10, lines 3-5; Page 15, lines 7-10; Page 56, lines 21-23). More particularly, the '216 application discloses that an agonistic Apo-2 antibody may be employed to activate or stimulate apoptosis in mammalian cancer cells (Page 56, lines 21-23). Methods for making Apo-2 antibodies are described on pages 48-56 of the '216 application. Apoptotic activity in mammalian cells is described on, e.g., page 17, lines 1-12, of the '216 application.

7. The '216 application therefore demonstrates that agonist antibodies which bind Apo-2 receptor and stimulate apoptosis were conceived and constructively reduced to practice by the May 15, 1997 filing date of my patent application.

8. I have read and reviewed US Patent 6,313,269 issued to Deen et al. on June 6, 2000 (hereinafter the "'269 patent") (a copy of which is attached as Exhibit B). I understand that the '269 patent is based on application serial no. 09/333,593 filed with the Patent Office on June 15, 1999 and claims priority to three earlier filed applications. I note that in these applications, the receptor sequence is termed "TR6."

9. I have read and reviewed the three applications referenced in the previous paragraph and to which the '269 patent claims priority. They are: 08/916,625 filed August 22, 1997 (attached as Exhibit C); 08/853,684 filed May 9, 1997 (attached as Exhibit D); and 60/041,230 filed March 14, 1997 (attached as Exhibit E). A depiction of the family is shown below, relative to the filing date of the '216 application.



10. Two of the three applications noted in the preceding paragraph were filed prior to the May 15, 1997 filing date of my '216 application. These are 08/853,684 (the '684 application) and 60/041,230 (the '230 application).

11. The only text appearing in the '269 patent concerning "potential TR6 agonists" that could be an antibody is contained in a single sentence that appears at Column 24, lines 57-60, and reads as follows:

> Examples of potential TR6 agonists include antibodies that bind to TR6, its ligand, such as TL2, or derivatives thereof, or small molecules that bind to TR6.

This sentence is not found in either the '684 or the '230 applications.

12. In comparing the disclosures of the '269 patent and its three priority applications, I found that the text referred to in paragraph 11 above was added for the first time in the '625 application, which was filed August 22, 1997 (i.e., after the filing date of the '216 application).

13. The '230 application discloses a polynucleotide sequence that encodes a 307 amino acid "partial" TR6 sequence. See, e.g., page 8, lines 25-28. The '230 application postulates that TR6 is structurally related to other proteins in the Tumor Necrosis Factor (TNF) receptor superfamily based on sequence homologies.

See, e.g., page 8, lines 25-33. The '230 application suggests that the disclosed TR6 sequence has about 25.0% homology with murine TNF-R2, 25.6% homology with human TNF-R1, and 21.3% homology with human TNF-R2.

14. The '230 application also postulates that the TR6 sequence contains a death domain (amino acids 220-277) based on a homology analysis to other members of the TNF receptor superfamily. See, e.g., page 9, lines 1-6. The application discloses that the TR6 protein has a 35.7% homology with the human DR3 death domain, 32.7% homology with the human TNF-R1 death domain, and 19.6% homology with the Fas death domain.

15. Based on these homology observations, the '230 application postulates the full-length TR6 is approximately 410 amino acids. See, page 9, lines 7-8.

16. The polynucleotide encoding the full-length (411 amino acids) TR6 polypeptide was not disclosed until the filing of the '684 application. See, e.g., page 10, line 23. The '684 application postulates that the full-length TR6, like the partial sequence disclosed in the '230 application, is structurally related to other proteins in the TNF receptor superfamily based on homology analyses to certain members of that superfamily. For instance, the '684 application postulates that TR6 has a 58% homology to human DR4 over 411 amino acids, and that TR6 has a death domain sequence (amino acids 290-324) based on homology analyses to certain members of the TNF receptor superfamily. See, p. 20, lines 28-29. The application discloses that the postulated death domain sequence of TR6 has 64% homology to the death domain of human DR4, 35.7% homology to the death domain of human DR3, 32.7% homology to the death domain of human TNF-R1, and 19.6% homology to the death domain of Fas. See, page 10, lines 29-30 and page 11, lines 1-5.

17. Neither the '684 nor the '230 applications provide any further analysis regarding the identity or conservation of specific amino acids within the putative death domain, which were known to be crucial for activity of the death domain of TNFR1 (see, e.g., Table 2, Tartaglia et al., Cell, 74, 845-853 (1993); Fig. 4B, Brojatsch et al., Cell, 87, 845-855 (1996)).

18. I note that the functional complexity of TNF receptor superfamily members that contain death domain motifs was well known in the art at the time of the filing of the '230 application, particularly with respect to the biological functions associated with binding of ligands to such receptors.

The '684 and '230 applications acknowledge that members of the TNF ligand or TNF receptor superfamily are known to have a wide and divergent range of biological activities and functions. See, e.g., '230 application at page 2, lines 27-28 and page 3, lines 3-8 and '684 application at page 2, lines 24-25 and page 3, lines 2-7. One example is the low affinity NGF receptor (p75 NGFR, also called "neurotrophin receptor" or "NTR") known prior to the filing date of the '684 and '230 applications. In Rabizadeh et al., Science, 261, 345-348 (1993), the authors teach that "expression of p75 NGFR induced neural cell death constitutively when p75 NGFR was unbound; binding by NGF or monoclonal antibody, however, inhibited cell death induced by p75 NGFR". In Chapman, FEBS Lett., 374, 216-220 (1995), the author states that "Unlike TNFR-1 and Fas, cell death induced by NTR (namely p75 NGFR) is reversed rather than caused by ligand binding". Thus, at the time of the filing of the '230 application, binding of ligand to NTR was known to inhibit, rather than stimulate, apoptosis. Therefore, the mere presence of a death domain related sequence is not, standing alone, indicative of the specific function or functions of a receptor in the TNF receptor superfamily, particularly those functions associated with ligand binding to such receptor.

19. Neither the '684 nor the '230 application provide any experimental data characterizing the expression product(s) of the disclosed polynucleotide sequence, or any data characterizing functions associated with ligand binding to such sequences.

20. The '684 and '230 applications, p. 30, line 16 and p. 20, line 20, respectively, indicate that through Northern Blot analysis varying levels of expression of TR6 was observed in aortic endothelial cells, monocytes, bone marrow, CD4+ activated PBLs, CD19+ PBLs, CD8+ PBLs (both activated and unstimulated), unstimulated CD4+ PBLsm, and in hematopoietic cell lines. A disclosure of such a wide expression pattern does not provide sufficient information to a person skilled in the art to suggest any specific activity or function for the molecule encoded by the disclosed TR6 sequences.

21. Neither the '684 nor the '230 application identify the ligand that specifically binds to the putative TR6 receptor. The '625 application (filed on August 22, 1997) was the first application in this family of applications to disclose that TRAIL is a ligand of the putative TR6 receptor.

22. For at least these reasons, I believe the '684 and '230 applications do not and cannot provide an adequate basis to

determine the specific biological functions of the putative TR6 receptor disclosed in those applications. In particular, it is my opinion that a person of ordinary skill in this field would not be able to determine, in view of the relatively low homology of the putative death domain described in the specifications, the absence of any description of the ligand that binds the putative receptor, and the lack of experimental data characterizing the expression product of the TR6 sequence, that the TR6 receptor mediates specific biological functions, such as apoptosis, upon ligand binding.

23. Also in light of the above facts and observations, it is my opinion that with respect to the '684 and '230 applications that one skilled in the art would find no suggestion to produce antibodies raised against the TR6 receptor that induce specific biological functions (e.g., apoptosis) upon binding of such antibodies to the putative receptor.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

3/22/04
Date

Avi J. Ashkenazi, Ph.D.

PATENT DOCKET NO. P1101
EXPRESS MAIL NO: EM239524622US
MAILED: May 15, 1997

Apo-2 Receptor

FIELD OF THE INVENTION

The present invention relates generally to the identification, isolation, and recombinant production of novel polypeptides, designated herein as "Apo-2".

BACKGROUND OF THE INVENTION

Apoptosis or "Programmed Cell Death"

Control of cell numbers in mammals is believed to be determined, in part, by a balance between cell proliferation and cell death. One form of cell death, sometimes referred to as necrotic cell death, is typically characterized as a pathologic form of cell death resulting from some trauma or cellular injury. In contrast, there is another, "physiologic" form of cell death which usually proceeds in an orderly or controlled manner. This orderly or controlled form of cell death is often referred to as "apoptosis" [see, *e.g.*, Barr et al., Bio/Technology, 12:487-493 (1994); Steller et al., Science, 267:1445-1449 (1995)]. Apoptotic cell death naturally occurs in many physiological processes, including embryonic development and clonal selection in the immune system [Itoh et al., Cell, 66:233-243 (1991)]. Decreased levels of apoptotic cell death have been associated with a variety of pathological conditions, including cancer, lupus, and herpes virus infection [Thompson, Science, 267:1456-1462 (1995)]. Increased levels of apoptotic cell death may be associated with a variety of other pathological conditions, including AIDS, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, retinitis pigmentosa, cerebellar degeneration, aplastic anemia, myocardial infarction, stroke, reperfusion injury, and toxin-induced liver disease [see, Thompson, supra].

Apoptotic cell death is typically accompanied by one or more characteristic morphological and biochemical changes in cells, such as condensation of cytoplasm, loss of plasma membrane microvilli, segmentation of the nucleus, degradation of chromosomal DNA or loss of mitochondrial function. A variety of extrinsic and intrinsic signals are believed to trigger or induce such morphological and biochemical cellular changes [Raff, Nature, 356:397-400 (1992); Steller, supra; Sachs et al., Blood, 82:15 (1993)]. For instance, they can be triggered by hormonal stimuli, such as glucocorticoid hormones for immature thymocytes, as well as withdrawal of certain growth factors [Watanabe-Fukunaga et al., Nature, 356:314-317 (1992)]. Also, some identified oncogenes such as *myc*, *rel*, and *E1A*, and tumor suppressors, like *p53*, have been reported to have a role in inducing apoptosis. Certain chemotherapy drugs and some forms of radiation have likewise been observed to have apoptosis-inducing activity [Thompson, supra].

TNF Family of Cytokines

Various molecules, such as tumor necrosis factor-α ("TNF-α"), tumor necrosis factor-β ("TNF-β" or "lymphotoxin"), CD30 ligand, CD27 ligand, CD40 ligand, OX-40 ligand, 4-1BB ligand, Apo-1 ligand (also referred to as Fas ligand or CD95 ligand), and Apo-2 ligand (also referred to as TRAIL) have been identified as members of the tumor necrosis factor ("TNF") family of cytokines [See, e.g., Gruss and Dower, Blood, 85:3378-3404 (1995); Wiley et al., Immunity, 3:673-682 (1995); Pitti et al., J. Biol. Chem., 271:12687-12690 (1996)]. Among these molecules, TNF-α, TNF-β, CD30 ligand, 4-1BB ligand, Apo-1 ligand, and Apo-2 ligand (TRAIL) have been reported to be involved in apoptotic cell death. Both TNF-α and TNF-β have been reported to induce apoptotic death in susceptible tumor cells [Schmid et al., Proc. Natl. Acad. Sci., 83:1881 (1986); Dealtry et al., Eur. J. Immunol., 17:689 (1987)]. Zheng et al. have reported that TNF-α is involved in post-stimulation apoptosis of CD8-positive T cells [Zheng et al., Nature, 377:348-351 (1995)]. Other investigators have reported that CD30 ligand may be involved in deletion of self-reactive T cells in the thymus [Amakawa et al., Cold Spring Harbor Laboratory

Symposium on Programmed Cell Death, Abstr. No. 10, (1995)].

Mutations in the mouse Fas/Apo-1 receptor or ligand genes (called *lpr* and *gld*, respectively) have been associated with some autoimmune disorders, indicating that Apo-1 ligand may play a role in regulating the clonal deletion of self-reactive lymphocytes in the periphery [Krammer et al., Curr. Op. Immunol., 6:279-289 (1994); Nagata et al., Science, 267:1449-1456 (1995)]. Apo-1 ligand is also reported to induce post-stimulation apoptosis in CD4-positive T lymphocytes and in B lymphocytes, and may be involved in the elimination of activated lymphocytes when their function is no longer needed [Krammer et al., supra; Nagata et al., supra]. Agonist mouse monoclonal antibodies specifically binding to the Apo-1 receptor have been reported to exhibit cell killing activity that is comparable to or similar to that of TNF-α [Yonehara et al., J. Exp. Med., 169:1747-1756 (1989)].

TNF Family of Receptors

Induction of various cellular responses mediated by such TNF family cytokines is believed to be initiated by their binding to specific cell receptors. Two distinct TNF receptors of approximately 55-kDa (TNFR1) and 75-kDa (TNFR2) have been identified [Hohman et al., J. Biol. Chem., 264:14927-14934 (1989); Brockhaus et al., Proc. Natl. Acad. Sci., 87:3127-3131 (1990); EP 417,563, published March 20, 1991] and human and mouse cDNAs corresponding to both receptor types have been isolated and characterized [Loetscher et al., Cell, 61:351 (1990); Schall et al., Cell, 61:361 (1990); Smith et al., Science, 248:1019-1023 (1990); Lewis et al., Proc. Natl. Acad. Sci., 88:2830-2834 (1991); Goodwin et al., Mol. Cell. Biol., 11:3020-3026 (1991)]. Extensive polymorphisms have been associated with both TNF receptor genes [see, e.g., Takao et al., Immunogenetics, 37:199-203 (1993)]. Both TNFRs share the typical structure of cell surface receptors including extracellular, transmembrane and intracellular regions. The extracellular portions of both receptors are found naturally also as soluble TNF-binding proteins [Nophar, Y. et al., EMBO J., 9:3269 (1990); and Kohno, T. et al., Proc. Natl. Acad. Sci. U.S.A.,

87:8331 (1990)]. More recently, the cloning of recombinant soluble TNF receptors was reported by Hale et al. [J. Cell. Biochem. Supplement 15F, 1991, p. 113 (P424)].

The extracellular portion of type 1 and type 2 TNFRs (TNFR1 and TNFR2) contains a repetitive amino acid sequence pattern of four cysteine-rich domains (CRDs) designated 1 through 4, starting from the NH_2-terminus. Each CRD is about 40 amino acids long and contains 4 to 6 cysteine residues at positions which are well conserved [Schall et al., supra; Loetscher et al., supra; Smith et al., supra; Nophar et al., supra; Kohno et al., supra]. In TNFR1, the approximate boundaries of the four CRDs are as follows: CRD1- amino acids 14 to about 53; CRD2- amino acids from about 54 to about 97; CRD3- amino acids from about 98 to about 138; CRD4- amino acids from about 139 to about 167. In TNFR2, CRD1 includes amino acids 17 to about 54; CRD2- amino acids from about 55 to about 97; CRD3- amino acids from about 98 to about 140; and CRD4- amino acids from about 141 to about 179 [Banner et al., Cell, 73:431-435 (1993)]. The potential role of the CRDs in ligand binding is also described by Banner et al., supra.

A similar repetitive pattern of CRDs exists in several other cell-surface proteins, including the p75 nerve growth factor receptor (NGFR) [Johnson et al., Cell, 47:545 (1986); Radeke et al., Nature, 325:593 (1987)], the B cell antigen CD40 [Stamenkovic et al., EMBO J., 8:1403 (1989)], the T cell antigen OX40 [Mallet et al., EMBO J., 9:1063 (1990)] and the Fas antigen [Yonehara et al., supra and Itoh et al., supra]. CRDs are also found in the soluble TNFR (sTNFR)-like T2 proteins of the Shope and myxoma poxviruses [Upton et al., Virology, 160:20-29 (1987); Smith et al., Biochem. Biophys. Res. Commun., 176:335 (1991); Upton et al., Virology, 184:370 (1991)]. Optimal alignment of these sequences indicates that the positions of the cysteine residues are well conserved. These receptors are sometimes collectively referred to as members of the TNF/NGF receptor superfamily. Recent studies on p75NGFR showed that the deletion of CRD1 [Welcher, A.A. et al., Proc. Natl. Acad. Sci. USA, 88:159-163 (1991)] or a 5-amino acid insertion in

this domain [Yan, H. and Chao, M.V., J. Biol. Chem., 266:12099-12104 (1991)] had little or no effect on NGF binding [Yan, H. and Chao, M.V., supra]. p75 NGFR contains a proline-rich stretch of about 60 amino acids, between its CRD4 and transmembrane region, which is not involved in NGF binding [Peetre, C. et al., Eur. J. Hematol., 41:414-419 (1988); Seckinger, P. et al., J. Biol. Chem., 264:11966-11973 (1989); Yan, H. and Chao, M.V., supra]. A similar proline-rich region is found in TNFR2 but not in TNFR1.

Itoh et al. disclose that the Apo-1 receptor can signal an apoptotic cell death similar to that signaled by the 55-kDa TNFR1 [Itoh et al., supra]. Expression of the Apo-1 antigen has also been reported to be down-regulated along with that of TNFR1 when cells are treated with either TNF-α or anti-Apo-1 mouse monoclonal antibody [Krammer et al., supra; Nagata et al., supra]. Accordingly, some investigators have hypothesized that cell lines that co-express both Apo-1 and TNFR1 receptors may mediate cell killing through common signaling pathways [Id.].

The TNF family ligands identified to date, with the exception of lymphotoxin-α, are type II transmembrane proteins, whose C-terminus is extracellular. In contrast, the receptors in the TNF receptor (TNFR) family identified to date are type I transmembrane proteins. In both the TNF ligand and receptor families, however, homology identified between family members has been found mainly in the extracellular domain ("ECD"). Several of the TNF family cytokines, including TNF-α, Apo-1 ligand and CD40 ligand, are cleaved proteolytically at the cell surface; the resulting protein in each case typically forms a homotrimeric molecule that functions as a soluble cytokine. TNF receptor family proteins are also usually cleaved proteolytically to release soluble receptor ECDs that can function as inhibitors of the cognate cytokines.

Recently, other members of the TNFR family have been identified. In Marsters et al., Curr. Biol., 6:750 (1996), investigators describe a full length native sequence human polypeptide, called Apo-3, which exhibits similarity to the TNFR

family in its extracellular cysteine-rich repeats and resembles TNFR1 and CD95 in that it contains a cytoplasmic death domain sequence [see also Marsters et al., Curr. Biol., 6:1669 (1996)]. Apo-3 has also been referred to by other investigators as DR3, wsl-1 and TRAMP [Chinnaiyan et al., Science, 274:990 (1996); Kitson et al., Nature, 384:372 (1996); Bodmer et al., Immunity, 6:79 (1997)].

Pan et al. have disclosed another TNF receptor family member referred to as "DR4" [Pan et al., Science, 276:111-113 (1997)]. The DR4 was reported to contain a cytoplasmic death domain capable of engaging the cell suicide apparatus. Pan et al. disclose that DR4 is believed to be a receptor for the ligand known as Apo-2 ligand or TRAIL.

The Apoptosis-Inducing Signaling Complex

As presently understood, the cell death program contains at least three important elements - activators, inhibitors, and effectors; in *C. elegans*, these elements are encoded respectively by three genes, *Ced-4*, *Ced-9* and *Ced-3* [Steller, Science, 267:1445 (1995); Chinnaiyan et al., Science, 275:1122-1126 (1997)]. Two of the TNFR family members, TNFR1 and Fas/Apo1 (CD95), can activate apoptotic cell death [Chinnaiyan and Dixit, Current Biology, 6:555-562 (1996); Fraser and Evan, Cell; 85:781-784 (1996)]. TNFR1 is also known to mediate activation of the transcription factor, NF-κB [Tartaglia et al., Cell, 74:845-853 (1993); Hsu et al., Cell, 84:299-308 (1996)]. In addition to some ECD homology, these two receptors share homology in their intracellular domain (ICD) in an oligomerization interface known as the death domain [Tartaglia et al., supra; Nagata, Cell, 88:355 (1997)]. Death domains are also found in several metazoan proteins that regulate apoptosis, namely, the Drosophila protein, Reaper, and the mammalian proteins referred to as FADD/MORT1, TRADD, and RIP [Cleaveland and Ihle, Cell, 81:479-482 (1995)]. Using the yeast-two hybrid system, Raven et al. report the identification of protein, wsl-1, which binds to the TNFR1 death domain [Raven et al., Programmed Cell Death Meeting, September 20-24, 1995, Abstract at page 127; Raven et al., European Cytokine Network, 7:Abstr. 82 at page 210 (April-June 1996)]. The

wsl-1 protein is described as being homologous to TNFR1 (48% identity) and having a restricted tissue distribution. According to Raven et al., the tissue distribution of wsl-1 is significantly different from the TNFR1 binding protein, TRADD.

Upon ligand binding and receptor clustering, TNFR1 and CD95 are believed to recruit FADD into a death-inducing signalling complex. CD95 purportedly binds FADD directly, while TNFR1 binds FADD indirectly via TRADD [Chinnaiyan et al., Cell, 81:505-512 (1995); Boldin et al., J. Biol. Chem., 270:387-391 (1995); Hsu et al., supra; Chinnaiyan et al., J. Biol. Chem., 271:4961-4965 (1996)]. It has been reported that FADD serves as an adaptor protein which recruits the *Ced-3*-related protease, MACHα/FLICE (caspase 8), into the death signalling complex [Boldin et al., Cell, 85:803-815 (1996); Muzio et al., Cell, 85:817-827 (1996)]. MACHα/FLICE appears to be the trigger that sets off a cascade of apoptotic proteases, including the interleukin-1β converting enzyme (ICE) and CPP32/Yama, which may execute some critical aspects of the cell death programme [Fraser and Evan, supra].

It was recently disclosed that programmed cell death involves the activity of members of a family of cysteine proteases related to the *C. elegans* cell death gene, *ced-3*, and to the mammalian IL-1-converting enzyme, ICE. The activity of the ICE and CPP32/Yama proteases can be inhibited by the product of the cowpox virus gene, *crmA* [Ray et al., Cell, 69:597-604 (1992); Tewari et al., Cell, 81:801-809 (1995)]. Recent studies show that CrmA can inhibit TNFR1- and CD95-induced cell death [Enari et al., Nature, 375:78-81 (1995); Tewari et al., J. Biol. Chem., 270:3255-3260 (1995)].

As reviewed recently by Tewari et al., TNFR1, TNFR2 and CD40 modulate the expression of proinflammatory and costimulatory cytokines, cytokine receptors, and cell adhesion molecules through activation of the transcription factor, NF-κB [Tewari et al., Curr. Op. Genet. Develop., 6:39-44 (1996)]. NF-κB is the prototype of a family of dimeric transcription factors whose subunits contain conserved Rel regions [Verma et al., Genes Develop., 9:2723-2735

(1996); Baldwin, Ann. Rev. Immunol., 14:649-681 (1996)]. In its latent form, NF-κB is complexed with members of the IκB inhibitor family; upon inactivation of the IκB in response to certain stimuli, released NF-κB translocates to the nucleus where it binds to specific DNA sequences and activates gene transcription.

For a review of the TNF family of cytokines and their receptors, see Gruss and Dower, supra.

SUMMARY OF THE INVENTION

Applicants have identified cDNA clones that encode novel polypeptides, designated in the present application as "Apo-2." It is believed that Apo-2 is a member of the TNFR family; full-length native sequence human Apo-2 polypeptide exhibits some similarities to some known TNFRs, including a cytoplasmic death domain region. Full-length native sequence human Apo-2 also exhibits similarity to the TNFR family in its extracellular cysteine-rich repeats. Apo-2 polypeptide has been found to be capable of triggering caspase-dependent apoptosis and activating NF-κB. Applicants surprisingly found that the soluble extracellular domain of Apo-2 binds Apo-2 ligand (Apo-2L) and can inhibit Apo-2 ligand function. It is presently believed that Apo-2 ligand can signal via at least two different receptors, DR4 and the newly described Apo-2 herein.

In one embodiment, the invention provides isolated Apo-2 polypeptide. In particular, the invention provides isolated native sequence Apo-2 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 1 to 411 of Figure 1 (SEQ ID NO:1). In other embodiments, the isolated Apo-2 polypeptide comprises at least about 80% amino acid sequence identity with native sequence Apo-2 polypeptide comprising residues 1 to 411 of Figure 1 (SEQ ID NO:1).

In another embodiment, the invention provides an isolated extracellular domain (ECD) sequence of Apo-2. Optionally, the isolated extracellular domain sequence comprises amino acid residues 54 to 182 of Fig. 1 (SEQ ID NO:1).

In another embodiment, the invention provides an isolated

death domain sequence of Apo-2. Optionally, the isolated death domain sequence comprises amino acid residues 324 to 391 of Fig. 1 (SEQ ID NO:1).

In another embodiment, the invention provides chimeric molecules comprising Apo-2 polypeptide fused to a heterologous polypeptide or amino acid sequence. An example of such a chimeric molecule comprises an Apo-2 fused to an immunoglobulin sequence. Another example comprises an extracellular domain sequence of Apo-2 fused to a heterologous polypeptide or amino acid sequence, such as an immunoglobulin sequence.

In another embodiment, the invention provides an isolated nucleic acid molecule encoding Apo-2 polypeptide. In one aspect, the nucleic acid molecule is RNA or DNA that encodes an Apo-2 polypeptide or a particular domain of Apo-2, or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions. In one embodiment, the nucleic acid sequence is selected from:

(a) the coding region of the nucleic acid sequence of Figure 1 (SEQ ID NO:2) that codes for residue 1 to residue 411 (i.e., nucleotides 140-142 through 1370-1372), inclusive;

(b) the coding region of the nucleic acid sequence of Figure 1 (SEQ ID NO:2) that codes for residue 1 to residue 182 (i.e., nucleotides 140-142 through 683-685), inclusive;

(c) the coding region of the nucleic acid sequence of Figure 1 (SEQ ID NO:2) that codes for residue 54 to residue 182 (i.e., nucleotides 299-301 through 683-685), inclusive;

(d) the coding region of the nucleic acid sequence of Figure 1 (SEQ ID NO:2) that codes for residue 324 to residue 391 (i.e., nucleotides 1109-1111 through 1310-1312), inclusive; or

(e) a sequence corresponding to the sequence of (a), (b), (c) or (d) within the scope of degeneracy of the genetic code.

In a further embodiment, the invention provides a vector comprising the nucleic acid molecule encoding the Apo-2 polypeptide or particular domain of Apo-2. A host cell comprising the vector

or the nucleic acid molecule is also provided. A method of producing Apo-2 is further provided.

In another embodiment, the invention provides an antibody which specifically binds to Apo-2. The antibody may be an agonistic, antagonistic or neutralizing antibody.

In another embodiment, the invention provides non-human, transgenic or knock-out animals.

A further embodiment of the invention provides articles of manufacture and kits that include Apo-2 or Apo-2 antibodies.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleotide sequence of a native sequence human Apo-2 cDNA and its derived amino acid sequence.

Figure 2A shows the derived amino acid sequence of a native sequence human Apo-2 - the putative signal sequence is underlined, the putative transmembrane domain is boxed, and the putative death domain sequence is dash underlined. The cysteines of the two cysteine-rich domains are individually underlined.

Figure 2B shows an alignment and comparison of the death domain sequences of native sequence human Apo-2, DR4, Apo-3/DR3, TNFR1, and Fas/Apo-1 (CD95). Asterisks indicate residues that are essential for death signaling by TNFR1 [Tartaglia et al., supra].

Figure 3 shows the interaction of the Apo-2 ECD with Apo-2L. Supernatants from mock-transfected 293 cells or from 293 cells transfected with Flag epitope-tagged Apo-2 ECD were incubated with poly-His-tagged Apo-2L and subjected to immunoprecipitation with anti-Flag conjugated or Nickel conjugated agarose beads. The precipitated proteins were resolved by electrophoresis on polyacrylamide gels, and detected by immunoblot with anti-Apo-2L or anti-Flag antibody.

Figure 4 shows the induction of apoptosis by Apo-2 and inhibition of Apo-2L activity by soluble Apo-2 ECD. Human 293 cells (A, B) or HeLa cells (C) were transfected by pRK5 vector or by pRK5-based plasmids encoding Apo-2 and/or CrmA. Apoptosis was assessed by morphology (A), DNA fragmentation (B), or by FACS (C-

E). Soluble Apo-2L was pre-incubated with buffer or affinity-purified Apo-2 ECD together with anti-Flag antibody or Apo-2 ECD immunoadhesin or DR4 or TNFR1 immunoadhesins and added to HeLa cells. The cells were later analyzed for apoptosis (D). Dose-response analysis using Apo-2L with Apo-2 ECD immunoadhesin was also determined (E).

Figure 5 shows activation of NF-κB by Apo-2, DR4, and Apo-2L. (A) HeLa cells were transfected with expression plasmids encoding the indicated proteins. Nuclear extracts were prepared and analyzed by an electrophoretic mobility shift assay. (B) HeLa cells or MCF7 cells were treated with buffer, Apo-2L or TNF-alpha and assayed for NF-κB activity. (C) HeLa cells were preincubated with buffer, ALLN or cyclohexamide before addition of Apo-2L. Apoptosis was later analyzed by FACS.

Figure 6 shows expression of Apo-2 mRNA in human tissues as analyzed by Northern hybridization of human tissue poly A RNA blots.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. Definitions

The terms "Apo-2 polypeptide" and "Apo-2" when used herein encompass native sequence Apo-2 and Apo-2 variants (which are further defined herein). These terms encompass Apo-2 from a variety of mammals, including humans. The Apo-2 may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods.

A "native sequence Apo-2" comprises a polypeptide having the same amino acid sequence as an Apo-2 derived from nature. Thus, a native sequence Apo-2 can have the amino acid sequence of naturally-occurring Apo-2 from any mammal. Such native sequence Apo-2 can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence Apo-2" specifically encompasses naturally-occurring truncated or secreted forms of the Apo-2 (*e.g.*, an extracellular domain sequence), naturally-occurring variant forms (*e.g.*, alternatively spliced forms) and naturally-

occurring allelic variants of the Apo-2. A naturally-occurring variant form of the Apo-2 includes an Apo-2 having an amino acid substitution at residue 410 in the amino acid sequence shown in Figure 1 (SEQ ID NO:1). In one embodiment of such naturally-ocurring variant form, the leucine residue at position 410 is substituted by a methionine residue. In Fig. 1 (SEQ ID NO:1), the amino acid residue at position 410 is identified as "Xaa" to indicate that the amino acid may, optionally, be either leucine or methionine. In Fig. 1 (SEQ ID NO:2), the nucleotide at position 1367 is identified as "W" to indicate that the nucleotide may be either adenine (A) or thymine (T) or uracil (U). In one embodiment of the invention, the native sequence Apo-2 is a mature or full-length native sequence Apo-2 comprising amino acids 1 to 411 of Fig. 1 (SEQ ID NO:1).

The "Apo-2 extracellular domain" or "Apo-2 ECD" refers to a form of Apo-2 which is essentially free of the transmembrane and cytoplasmic domains of Apo-2. Ordinarily, Apo-2 ECD will have less than 1% of such transmembrane and/or cytoplasmic domains and preferably, will have less than 0.5% of such domains. Optionally, Apo-2 ECD will comprise amino acid residues 54 to 182 of Fig. 1 (SEQ ID NO:1) or amino acid residues 1 to 182 of Fig. 1 (SEQ ID NO:1).

"Apo-2 variant" means a biologically active Apo-2 as defined below having at least about 80% amino acid sequence identity with the Apo-2 having the deduced amino acid sequence shown in Fig. 1 (SEQ ID NO:1) for a full-length native sequence human Apo-2. Such Apo-2 variants include, for instance, Apo-2 polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of the sequence of Fig. 1 (SEQ ID NO:1). Ordinarily, an Apo-2 variant will have at least about 80% amino acid sequence identity, more preferably at least about 90% amino acid sequence identity, and even more preferably at least about 95% amino acid sequence identity with the amino acid sequence of Fig. 1 (SEQ ID NO:1).

"Percent (%) amino acid sequence identity" with respect

to the Apo-2 sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the Apo-2 sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as ALIGN™ or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising Apo-2, or a domain sequence thereof, fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the Apo-2. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 to about 50 amino acid residues (preferably, between about 10 to about 20 residues).

"Isolated," when used to describe the various polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning

cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the Apo-2 natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

An "isolated" Apo-2 nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the Apo-2 nucleic acid. An isolated Apo-2 nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated Apo-2 nucleic acid molecules therefore are distinguished from the Apo-2 nucleic acid molecule as it exists in natural cells. However, an isolated Apo-2 nucleic acid molecule includes Apo-2 nucleic acid molecules contained in cells that ordinarily express Apo-2 where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that

the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

The term "antibody" is used in the broadest sense and specifically covers single anti-Apo-2 monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies) and anti-Apo-2 antibody compositions with polyepitopic specificity.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen.

The monoclonal antibodies herein include hybrid and recombinant antibodies produced by splicing a variable (including hypervariable) domain of an anti-Apo-2 antibody with a constant domain (*e.g.* "humanized" antibodies), or a light chain with a heavy chain, or a chain from one species with a chain from another species, or fusions with heterologous proteins, regardless of species of origin or immunoglobulin class or subclass designation, as well as antibody fragments (*e.g.*, Fab, $F(ab')_2$, and Fv), so long as they exhibit the desired biological activity. See, *e.g.* U.S. Pat. No. 4,816,567 and Mage et al., in Monoclonal Antibody Production Techniques and Applications, pp.79-97 (Marcel Dekker, Inc.: New York, 1987).

Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous

population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, Nature, 256:495 (1975), or may be made by recombinant DNA methods such as described in U.S. Pat. No. 4,816,567. The "monoclonal antibodies" may also be isolated from phage libraries generated using the techniques described in McCafferty et al., Nature, 348:552-554 (1990), for example.

"Humanized" forms of non-human (*e.g.* murine) antibodies are specific chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized antibody may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin.

"Biologically active" and "desired biological activity" for the purposes herein mean having the ability to modulate

apoptosis (either in an agonistic or stimulating manner or in an antagonistic or blocking manner) in at least one type of mammalian cell *in vivo* or *ex vivo*.

The terms "apoptosis" and "apoptotic activity" are used in a broad sense and refer to the orderly or controlled form of cell death in mammals that is typically accompanied by one or more characteristic cell changes, including condensation of cytoplasm, loss of plasma membrane microvilli, segmentation of the nucleus, degradation of chromosomal DNA or loss of mitochondrial function. This activity can be determined and measured, for instance, by cell viability assays, FACS analysis or DNA electrophoresis, all of which are known in the art.

The terms "treating," "treatment," and "therapy" as used herein refer to curative therapy, prophylactic therapy, and preventative therapy.

The term "mammal" as used herein refers to any mammal classified as a mammal, including humans, cows, horses, dogs and cats. In a preferred embodiment of the invention, the mammal is a human.

II. Compositions and Methods of the Invention

The present invention provides newly identified and isolated Apo-2 polypeptides. In particular, Applicants have identified and isolated various human Apo-2 polypeptides. The properties and characteristics of some of these Apo-2 polypeptides are described in further detail in the Examples below. Based upon the properties and characteristics of the Apo-2 polypeptides disclosed herein, it is Applicants' present belief that Apo-2 is a member of the TNFR family.

A description follows as to how Apo-2, as well as Apo-2 chimeric molecules and anti-Apo-2 antibodies, may be prepared.

A. Preparation of Apo-2

The description below relates primarily to production of Apo-2 by culturing cells transformed or transfected with a vector containing Apo-2 nucleic acid. It is of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare Apo-2.

1. Isolation of DNA Encoding Apo-2

The DNA encoding Apo-2 may be obtained from any cDNA library prepared from tissue believed to possess the Apo-2 mRNA and to express it at a detectable level. Accordingly, human Apo-2 DNA can be conveniently obtained from a cDNA library prepared from human tissues, such as the bacteriophage libraries of human pancreas and kidney cDNA described in Example 1. The Apo-2-encoding gene may also be obtained from a genomic library or by oligonucleotide synthesis.

Libraries can be screened with probes (such as antibodies to the Apo-2 or oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described in Sambrook et al., Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the gene encoding Apo-2 is to use PCR methodology [Sambrook et al., supra; Dieffenbach et al., PCR Primer:A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1995)].

A preferred method of screening employs selected oligonucleotide sequences to screen cDNA libraries from various human tissues. Example 1 below describes techniques for screening a cDNA library. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The oligonucleotide is preferably labeled such that it can be detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like ^{32}P-labeled ATP, biotinylation or enzyme labeling. Hybridization conditions,

inlcuding moderate stringency and high stringency, are provided in Sambrook et al., supra.

Nucleic acid having all the protein coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in Sambrook et al., supra, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

Apo-2 variants can be prepared by introducing appropriate nucleotide changes into the Apo-2 DNA, or by synthesis of the desired Apo-2 polypeptide. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of the Apo-2, such as changing the number or position of glycosylation sites or altering the membrane anchoring characteristics.

Variations in the native full-length sequence Apo-2 or in various domains of the Apo-2 described herein, can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Pat. No. 5,364,934. Variations may be a substitution, deletion or insertion of one or more codons encoding the Apo-2 that results in a change in the amino acid sequence of the Apo-2 as compared with the native sequence Apo-2. Optionally the variation is by substitution of at least one amino acid with any other amino acid in one or more of the domains of the Apo-2 molecule. The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter et al., Nucl. Acids Res., 13:4331 (1986); Zoller et al., Nucl. Acids Res., 10:6487 (1987)], cassette mutagenesis [Wells et al., Gene, 34:315 (1985)], restriction selection mutagenesis [Wells et al., Philos. Trans. R. Soc. London SerA, 317:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the Apo-2 variant DNA.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence which are involved in the interaction with a particular ligand or receptor. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is the preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant. Alanine is also preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions [Creighton, The Proteins, (W.H. Freeman & Co., N.Y.); Chothia, J. Mol. Biol., 150:1 (1976)]. If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

Once selected Apo-2 variants are produced, they can be contacted with, for instance, Apo-2L, and the interaction, if any, can be determined. The interaction between the Apo-2 variant and Apo-2L can be measured by an *in vitro* assay, such as described in the Examples below. While any number of analytical measurments can be used to compare activities and properties between a native sequence Apo-2 and an Apo-2 variant, a convenient one for binding is the dissociation constant K_d of the complex formed between the Apo-2 variant and Apo-2L as compared to the K_d for the native sequence Apo-2. Generally, a $\geq$ 3-fold increase or decrease in K_d per substituted residue indicates that the substituted residue(s) is active in the interaction of the native sequence Apo-2 with the Apo-2L.

Optionally, representative sites in the Apo-2 sequence suitable for mutagenesis would include sites within the extracellular domain, and particularly, within one or both of the cysteine-rich domains. Such variations can be accomplished using the methods described above.

2. Insertion of Nucleic Acid into A Replicable Vector

The nucleic acid (*e.g.*, cDNA or genomic DNA) encoding Apo-2 may be inserted into a replicable vector for further cloning

(amplification of the DNA) or for expression. Various vectors are publicly available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence, each of which is described below.

(i) Signal Sequence Component

The Apo-2 may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the Apo-2 DNA that is inserted into the vector. The heterologous signal sequence selected preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, *e.g.*, the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders, the latter described in U.S. Pat. No. 5,010,182), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression the native Apo-2 presequence that normally directs insertion of Apo-2 in the cell membrane of human cells *in vivo* is satisfactory, although other mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders, for example, the herpes simplex glycoprotein D signal.

The DNA for such precursor region is preferably ligated in reading frame to DNA encoding Apo-2.

(ii) Origin of Replication Component

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used because it contains the early promoter).

Most expression vectors are "shuttle" vectors, *i.e.*, they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

DNA may also be amplified by insertion into the host genome. This is readily accomplished using *Bacillus* species as hosts, for example, by including in the vector a DNA sequence that is complementary to a sequence found in *Bacillus* genomic DNA. Transfection of *Bacillus* with this vector results in homologous recombination with the genome and insertion of Apo-2 DNA. However, the recovery of genomic DNA encoding Apo-2 is more complex than that of an exogenously replicated vector because restriction enzyme digestion is required to excise the Apo-2 DNA.

(iii) Selection Gene Component

Expression and cloning vectors typically contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host

cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*, the gene encoding D-alanine racemase for *Bacilli*.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin [Southern et al., J. Molec. Appl. Genet., 1:327 (1982)], mycophenolic acid (Mulligan et al., Science, 209:1422 (1980)] or hygromycin [Sugden et al., Mol. Cell. Biol., 5:410-413 (1985)]. The three examples given above employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid), or hygromycin, respectively.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the Apo-2 nucleic acid, such as DHFR or thymidine kinase. The mammalian cell transformants are placed under selection pressure that only the transformants are uniquely adapted to survive by virtue of having taken up the marker. Selection pressure is imposed by culturing the transformants under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to amplification of both the selection gene and the DNA that encodes Apo-2. Amplification is the process by which genes in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of Apo-2 are synthesized from the amplified DNA. Other examples of amplifiable genes include metallothionein-I and -II, adenosine deaminase, and

ornithine decarboxylase.

Cells transformed with the DHFR selection gene may first be identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub et al., Proc. Natl. Acad. Sci. USA, 77:4216 (1980). The transformed cells are then exposed to increased levels of methotrexate. This leads to the synthesis of multiple copies of the DHFR gene, and, concomitantly, multiple copies of other DNA comprising the expression vectors, such as the DNA encoding Apo-2. This amplification technique can be used with any otherwise suitable host, *e.g.*, ATCC No. CCL61 CHO-K1, notwithstanding the presence of endogenous DHFR if, for example, a mutant DHFR gene that is highly resistant to Mtx is employed (EP 117,060).

Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding Apo-2, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, *e.g.*, kanamycin, neomycin, or G418. See U.S. Patent No. 4,965,199.

A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 [Stinchcomb et al., Nature, 282:39 (1979); Kingsman et al., Gene, 7:141 (1979); Tschemper et al., Gene, 10:157 (1980)]. The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 [Jones, Genetics, 85:12 (1977)]. The presence of the *trp1* lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, *Leu2*-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the *Leu2* gene.

In addition, vectors derived from the 1.6 μm circular plasmid pKD1 can be used for transformation of *Kluyveromyces* yeasts [Bianchi et al., Curr. Genet., 12:185 (1987)]. More recently, an expression system for large-scale production of recombinant calf chymosin was reported for *K. lactis* [Van den Berg, Bio/Technology, 8:135 (1990)]. Stable multi-copy expression vectors for secretion of mature recombinant human serum albumin by industrial strains of *Kluyveromyces* have also been disclosed [Fleer et al., Bio/Technology, 9:968-975 (1991)].

(iv) Promoter Component

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the Apo-2 nucleic acid sequence. Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of particular nucleic acid sequence, such as the Apo-2 nucleic acid sequence, to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, *e.g.*, the presence or absence of a nutrient or a change in temperature. At this time a large number of promoters recognized by a variety of potential host cells are well known. These promoters are operably linked to Apo-2 encoding DNA by removing the promoter from the source DNA by restriction enzyme digestion and inserting the isolated promoter sequence into the vector. Both the native Apo-2 promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the Apo-2 DNA.

Promoters suitable for use with prokaryotic hosts include the β-lactamase and lactose promoter systems [Chang et al., Nature, 275:615 (1978); Goeddel et al., Nature, 281:544 (1979)], alkaline phosphatase, a tryptophan (trp) promoter system [Goeddel, Nucleic Acids Res., 8:4057 (1980); EP 36,776], and hybrid promoters such as the tac promoter [deBoer et al., Proc. Natl. Acad. Sci. USA, 80:21-

25 (1983)]. However, other known bacterial promoters are suitable. Their nucleotide sequences have been published, thereby enabling a skilled worker operably to ligate them to DNA encoding Apo-2 [Siebenlist et al., Cell, 20:269 (1980)] using linkers or adaptors to supply any required restriction sites. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding Apo-2.

Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into eukaryotic expression vectors.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman et al., J. Biol. Chem., 255:2073 (1980)] or other glycolytic enzymes [Hess et al., J. Adv. Enzyme Reg., 7:149 (1968); Holland, Biochemistry, 17:4900 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657. Yeast enhancers also are advantageously used with yeast promoters.

Apo-2 transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the Apo-2 sequence, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication [Fiers et al., Nature, 273:113 (1978); Mulligan and Berg, Science, 209:1422-1427 (1980); Pavlakis et al., Proc. Natl. Acad. Sci. USA, 78:7398-7402 (1981)]. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment [Greenaway et al., Gene, 18:355-360 (1982)]. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Patent No. 4,419,446. A modification of this system is described in U.S. Patent No. 4,601,978 [See also Gray et al., Nature, 295:503-508 (1982) on expressing cDNA encoding immune interferon in monkey cells; Reyes et al., Nature, 297:598-601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus; Canaani and Berg, Proc. Natl. Acad. Sci. USA 79:5166-5170 (1982) on expression of the human interferon β1 gene in cultured mouse and rabbit cells; and Gorman et al., Proc. Natl. Acad. Sci. USA, 79:6777-6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter].

(v) Enhancer Element Component

Transcription of a DNA encoding the Apo-2 of this

invention by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Enhancers are relatively orientation and position independent, having been found 5' [Laimins et al., Proc. Natl. Acad. Sci. USA, 78:993 (1981)] and 3' [Lusky et al., Mol. Cell Bio., 3:1108 (1983)] to the transcription unit, within an intron [Banerji et al., Cell, 33:729 (1983)], as well as within the coding sequence itself [Osborne et al., Mol. Cell Bio., 4:1293 (1984)]. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature, 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the Apo-2 coding sequence, but is preferably located at a site 5' from the promoter.

(vi) Transcription Termination Component

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding Apo-2.

(vii) Construction and Analysis of Vectors

Construction of suitable vectors containing one or more of the above-listed components employs standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and re-ligated in the form desired to generate the

plasmids required.

For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures can be used to transform *E. coli* K12 strain 294 (ATCC 31,446) and successful transformants selected by ampicillin or tetracycline resistance where appropriate. Plasmids from the transformants are prepared, analyzed by restriction endonuclease digestion, and/or sequenced by the method of Messing et al., Nucleic Acids Res., 9:309 (1981) or by the method of Maxam et al., Methods in Enzymology, 65:499 (1980).

(viii) Transient Expression Vectors

Expression vectors that provide for the transient expression in mammalian cells of DNA encoding Apo-2 may be employed. In general, transient expression involves the use of an expression vector that is able to replicate efficiently in a host cell, such that the host cell accumulates many copies of the expression vector and, in turn, synthesizes high levels of a desired polypeptide encoded by the expression vector [Sambrook et al., *supra*]. Transient expression systems, comprising a suitable expression vector and a host cell, allow for the convenient positive identification of polypeptides encoded by cloned DNAs, as well as for the rapid screening of such polypeptides for desired biological or physiological properties. Thus, transient expression systems are particularly useful in the invention for purposes of identifying Apo-2 variants.

(ix) Suitable Exemplary Vertebrate Cell Vectors

Other methods, vectors, and host cells suitable for adaptation to the synthesis of Apo-2 in recombinant vertebrate cell culture are described in Gething et al., Nature, 293:620-625 (1981); Mantei et al., Nature, 281:40-46 (1979); EP 117,060; and EP 117,058.

3. Selection and Transformation of Host Cells

Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes for this purpose include but are not limited to eubacteria, such as Gram-negative or

Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia, e.g., E. coli, Enterobacter, Erwinia, Klebsiella, Proteus, Salmonella, e.g., Salmonella typhimurium, Serratia, e.g., Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (*e.g., B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. Preferably, the host cell should secrete minimal amounts of proteolytic enzymes.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for Apo-2-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein.

Suitable host cells for the expression of glycosylated Apo-2 are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified [See, *e.g.*, Luckow et al., Bio/Technology, 6:47-55 (1988); Miller et al., in Genetic Engineering, Setlow et al., eds., Vol. 8 (Plenum Publishing, 1986), pp. 277-279; and Maeda et al., Nature, 315:592-594 (1985)]. A variety of viral strains for transfection are publicly available, *e.g.*, the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*. During incubation of the plant cell culture with *A. tumefaciens*, the DNA encoding the Apo-2

can be transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the Apo-2-encoding DNA. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences [Depicker et al., J. Mol. Appl. Gen., 1:561 (1982)]. In addition, DNA segments isolated from the upstream region of the T-DNA *780* gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue [EP 321,196 published 21 June 1989].

Propagation of vertebrate cells in culture (tissue culture) is also well known in the art [See, *e.g.*, Tissue Culture, Academic Press, Kruse and Patterson, editors (1973)]. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol., 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77:4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci., 383:44-68 (1982)); MRC 5 cells; and FS4 cells.

Host cells are transfected and preferably transformed with the above-described expression or cloning vectors for Apo-2 production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in

fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, $CaPO_4$ and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

Transformation means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook et al., supra, or electroporation is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw et al., Gene, 23:315 (1983) and WO 89/05859 published 29 June 1989. In addition, plants may be transfected using ultrasound treatment as described in WO 91/00358 published 10 January 1991.

For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) is preferred. General aspects of mammalian cell host system transformations have been described in U.S. Pat. No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen et al., J. Bact., 130:946 (1977) and Hsiao et al., Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, *e.g.*, polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown et al., Methods in Enzymology, 185:527-537 (1990) and Mansour et al., Nature, 336:348-352 (1988).

4. Culturing the Host Cells

Prokaryotic cells used to produce Apo-2 may be cultured in suitable media as described generally in Sambrook et al., supra.

The mammalian host cells used to produce Apo-2 may be

cultured in a variety of media. Examples of commercially available media include Ham's F10 (Sigma), Minimal Essential Medium ("MEM", Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ("DMEM", Sigma). Any such media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

In general, principles, protocols, and practical techniques for maximizing the productivity of mammalian cell cultures can be found in Mammalian Cell Biotechnology: a Practical Approach, M. Butler, ed. (IRL Press, 1991).

The host cells referred to in this disclosure encompass cells in culture as well as cells that are within a host animal.

5. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA [Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 (1980)], dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, and particularly ^{32}P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionucleotides, fluorescers or enzymes. Alternatively,

antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, or luminescent labels.

Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence Apo-2 polypeptide or against a synthetic peptide based on the DNA sequences provided herein or against exogenous sequence fused to Apo-2 DNA and encoding a specific antibody epitope.

6. Purification of Apo-2 Polypeptide

Forms of Apo-2 may be recovered from culture medium or from host cell lysates. If the Apo-2 is membrane-bound, it can be released from the membrane using a suitable detergent solution (*e.g.* Triton-X 100) or its extracellular domain may be released by enzymatic cleavage.

When Apo-2 is produced in a recombinant cell other than one of human origin, the Apo-2 is free of proteins or polypeptides of human origin. However, it may be desired to purify Apo-2 from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to Apo-2. As a first step, the culture medium or lysate may be centrifuged to remove particulate cell debris. Apo-2 thereafter is purified from

contaminant soluble proteins and polypeptides, with the following procedures being exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; and protein A Sepharose columns to remove contaminants such as IgG.

Apo-2 variants in which residues have been deleted, inserted, or substituted can be recovered in the same fashion as native sequence Apo-2, taking account of changes in properties occasioned by the variation. For example, preparation of an Apo-2 fusion with another protein or polypeptide, *e.g.*, a bacterial or viral antigen, immunoglobulin sequence, or receptor sequence, may facilitate purification; an immunoaffinity column containing antibody to the sequence can be used to adsorb the fusion polypeptide. Other types of affinity matrices also can be used.

A protease inhibitor such as phenyl methyl sulfonyl fluoride (PMSF) also may be useful to inhibit proteolytic degradation during purification, and antibiotics may be included to prevent the growth of adventitious contaminants. One skilled in the art will appreciate that purification methods suitable for native sequence Apo-2 may require modification to account for changes in the character of Apo-2 or its variants upon expression in recombinant cell culture.

7. Covalent Modifications of Apo-2 Polypeptides

Covalent modifications of Apo-2 are included within the scope of this invention. One type of covalent modification of the Apo-2 is introduced into the molecule by reacting targeted amino acid residues of the Apo-2 with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues of the Apo-2.

Derivatization with bifunctional agents is useful for crosslinking Apo-2 to a water-insoluble support matrix or surface for use in the method for purifying anti-Apo-2 antibodies, and

vice-versa. Derivatization with one or more bifunctional agents will also be useful for crosslinking Apo-2 molecules to generate Apo-2 dimers. Such dimers may increase binding avidity and extend half-life of the molecule *in vivo*. Commonly used crosslinking agents include, *e.g.*, 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidyl-propionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)-dithio]propioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates described in U.S. Patent Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

Other modifications include deamidation of glutaminyl and asparaginyl residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α-amino groups of lysine, arginine, and histidine side chains [T.E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)]; acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group. The modified forms of the residues fall within the scope of the present invention.

Another type of covalent modification of the Apo-2 polypeptide included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in native sequence Apo-2, and/or adding one or more glycosylation sites that are not present in the native sequence Apo-2.

Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxylamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the Apo-2 polypeptide may be accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the native sequence Apo-2 (for O-linked glycosylation sites). The Apo-2 amino acid sequence may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the Apo-2 polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids. The DNA mutation(s) may be made using methods described above and in U.S. Pat. No. 5,364,934, supra.

Another means of increasing the number of carbohydrate moieties on the Apo-2 polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330 published 11 September 1987, and in Aplin and Wriston, CRC Crit. Rev. Biochem., pp. 259-306 (1981).

Removal of carbohydrate moieties present on the Apo-2 polypeptide may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. For instance, chemical deglycosylation by exposing the polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound can result in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin, et al., Arch. Biochem. Biophys., 259:52 (1987) and by Edge et al., Anal. Biochem., 118:131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al., Meth. Enzymol., 138:350 (1987).

Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin et al., J. Biol. Chem., 257:3105 (1982). Tunicamycin blocks the formation of protein-N-glycoside linkages.

Another type of covalent modification of Apo-2 comprises linking the Apo-2 polypeptide to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

8. Apo-2 Chimeras

The present invention also provides chimeric molecules comprising Apo-2 fused to another, heterologous polypeptide or amino acid sequence.

In one embodiment, the chimeric molecule comprises a fusion of the Apo-2 with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl- terminus of the Apo-2. The presence of such epitope-tagged forms of the Apo-2 can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the Apo-2

to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag.

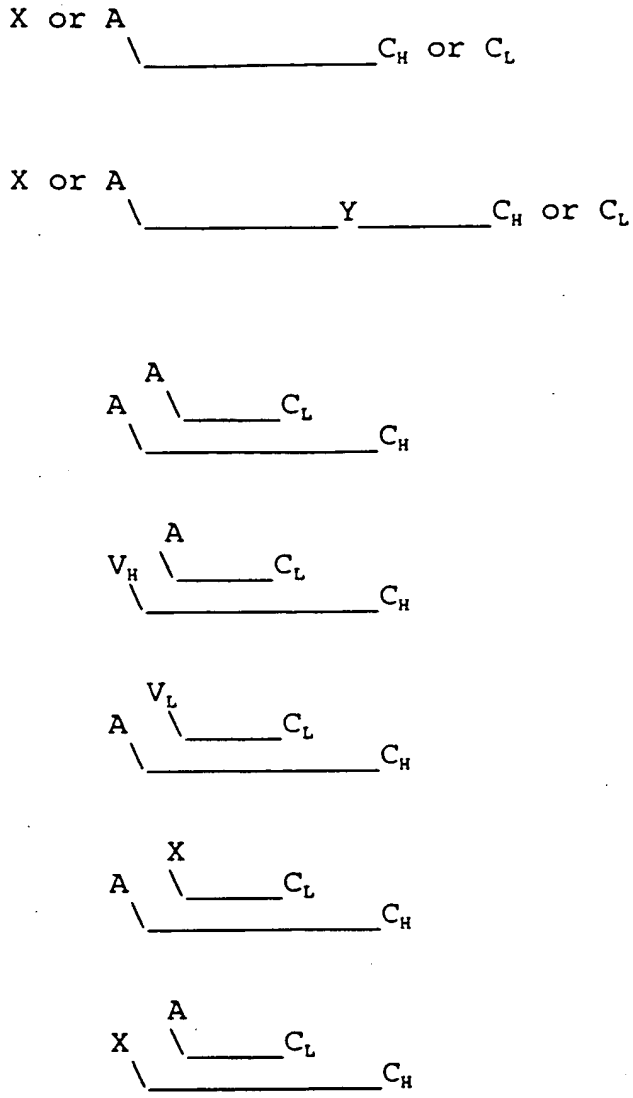
Various tag polypeptides and their respective antibodies are well known in the art. Examples include the flu HA tag polypeptide and its antibody 12CA5 [Field et al., Mol. Cell. Biol., 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al., Molecular and Cellular Biology, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky et al., Protein Engineering, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag-peptide [Hopp et al., BioTechnology, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin et al., Science, 255:192-194 (1992)]; an α-tubulin epitope peptide [Skinner et al., J. Biol. Chem., 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth et al., Proc. Natl. Acad. Sci. USA, 87:6393-6397 (1990)]. Once the tag polypeptide has been selected, an antibody thereto can be generated using the techniques disclosed herein.

Generally, epitope-tagged Apo-2 may be constructed and produced according to the methods described above. Epitope-tagged Apo-2 is also described in the Examples below. Apo-2-tag polypeptide fusions are preferably constructed by fusing the cDNA sequence encoding the Apo-2 portion in-frame to the tag polypeptide DNA sequence and expressing the resultant DNA fusion construct in appropriate host cells. Ordinarily, when preparing the Apo-2-tag polypeptide chimeras of the present invention, nucleic acid encoding the Apo-2 will be fused at its 3′ end to nucleic acid encoding the N-terminus of the tag polypeptide, however 5′ fusions are also possible. For example, a polyhistidine sequence of about 5 to about 10 histidine residues may be fused at the N- terminus or the C- terminus and used as a purification handle in affinity chromatography.

Epitope-tagged Apo-2 can be purified by affinity chromatography using the anti-tag antibody. The matrix to which

the affinity antibody is attached may include, for instance, agarose, controlled pore glass or poly(styrenedivinyl)benzene. The epitope-tagged Apo-2 can then be eluted from the affinity column using techniques known in the art.

In another embodiment, the chimeric molecule comprises an Apo-2 polypeptide fused to an immunoglobulin sequence. The chimeric molecule may also comprise a particular domain sequence of Apo-2, such as the extracellular domain sequence of native Apo-2 fused to an immunoglobulin sequence. This includes chimeras in monomeric, homo- or heteromultimeric, and particularly homo- or heterodimeric, or -tetrameric forms; optionally, the chimeras may be in dimeric forms or homodimeric heavy chain forms. Generally, these assembled immunoglobulins will have known unit structures as represented by the following diagrams.

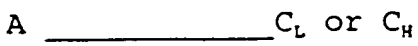


A basic four chain structural unit is the form in which IgG, IgD, and IgE exist. A four chain unit is repeated in the higher molecular weight immunoglobulins; IgM generally exists as a pentamer of basic four-chain units held together by disulfide bonds. IgA globulin, and occasionally IgG globulin, may also exist in a multimeric form in serum. In the case of multimers, each four chain unit may be the same or different.

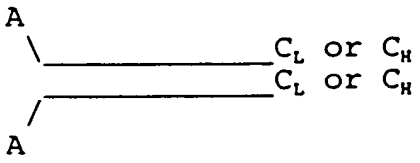
The following diagrams depict some exemplary monomer, homo- and heterodimer and homo- and heteromultimer structures. These diagrams are merely illustrative, and the chains of the

multimers are believed to be disulfide bonded in the same fashion as native immunoglobulins.

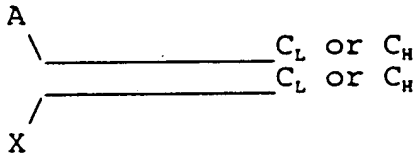
monomer:



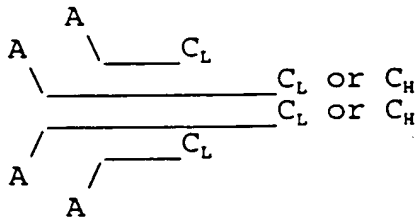
homodimer:



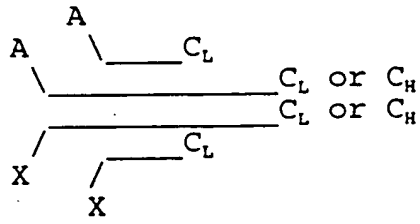
heterodimer:



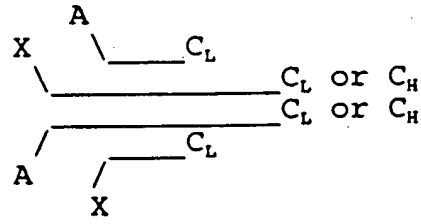
homotetramer:



heterotetramer:



and



In the foregoing diagrams, "A" means an Apo-2 sequence or an Apo-2 sequence fused to a heterologous sequence; X is an additional agent, which may be the same as A or different, a

portion of an immunoglobulin superfamily member such as a variable region or a variable region-like domain, including a native or chimeric immunoglobulin variable region, a toxin such a pseudomonas exotoxin or ricin, or a sequence functionally binding to another protein, such as other cytokines (i.e., IL-1, interferon-γ) or cell surface molecules (i.e., NGFR, CD40, OX40, Fas antigen, T2 proteins of Shope and myxoma poxviruses), or a polypeptide therapeutic agent not otherwise normally associated with a constant domain; Y is a linker or another receptor sequence; and V_L, V_H, C_L and C_H represent light or heavy chain variable or constant domains of an immunoglobulin. Structures comprising at least one CRD of an Apo-2 sequence as "A" and another cell-surface protein having a repetitive pattern of CRDs (such as TNFR) as "X" are specifically included.

It will be understood that the above diagrams are merely exemplary of the possible structures of the chimeras of the present invention, and do not encompass all possibilities. For example, there might desirably be several different "A"s, "X"s, or "Y"s in any of these constructs. Also, the heavy or light chain constant domains may be originated from the same or different immunoglobulins. All possible permutations of the illustrated and similar structures are all within the scope of the invention herein.

In general, the chimeric molecules can be constructed in a fashion similar to chimeric antibodies in which a variable domain from an antibody of one species is substituted for the variable domain of another species. See, for example, EP 0 125 023; EP 173,494; Munro, Nature, 312:597 (13 December 1984); Neuberger et al., Nature, 312:604-608 (13 December 1984); Sharon et al., Nature, 309:364-367 (24 May 1984); Morrison et al., Proc. Nat'l. Acad. Sci. USA, 81:6851-6855 (1984); Morrison et al., Science, 229:1202-1207 (1985); Boulianne et al., Nature, 312:643-646 (13 December 1984); Capon et al., Nature, 337:525-531 (1989); Traunecker et al., Nature, 339:68-70 (1989).

Alternatively, the chimeric molecules may be constructed

as follows. The DNA including a region encoding the desired sequence, such as an Apo-2 and/or TNFR sequence, is cleaved by a restriction enzyme at or proximal to the 3' end of the DNA encoding the immunoglobulin-like domain(s) and at a point at or near the DNA encoding the N-terminal end of the Apo-2 or TNFR polypeptide (where use of a different leader is contemplated) or at or proximal to the N-terminal coding region for TNFR (where the native signal is employed). This DNA fragment then is readily inserted proximal to DNA encoding an immunoglobulin light or heavy chain constant region and, if necessary, the resulting construct tailored by deletional mutagenesis. Preferably, the Ig is a human immunoglobulin when the chimeric molecule is intended for *in vivo* therapy for humans. DNA encoding immunoglobulin light or heavy chain constant regions is known or readily available from cDNA libraries or is synthesized. See for example, Adams et al., Biochemistry, 19:2711-2719 (1980); Gough et al., Biochemistry, 19:2702-2710 (1980); Dolby et al., Proc. Natl. Acad. Sci.. USA, 77:6027-6031 (1980); Rice et al., Proc. Natl. Acad. Sci., 79:7862-7865 (1982); Falkner et al., Nature, 298:286-288 (1982); and Morrison et al., Ann. Rev. Immunol., 2:239-256 (1984).

Further details of how to prepare such fusions are found in publications concerning the preparation of immunoadhesins. Immunoadhesins in general, and CD4-Ig fusion molecules specifically are disclosed in WO 89/02922, published 6 April 1989). Molecules comprising the extracellular portion of CD4, the receptor for human immunodeficiency virus (HIV), linked to IgG heavy chain constant region are known in the art and have been found to have a markedly longer half-life and lower clearance than the soluble extracellular portion of CD4 [Capon et al., supra; Byrn et al., Nature, 344:667 (1990)]. The construction of specific chimeric TNFR-IgG molecules is also described in Ashkenazi et al. Proc. Natl. Acad. Sci., 88:10535-10539 (1991); Lesslauer et al. [J. Cell. Biochem. Supplement 15F, 1991, p. 115 (P 432)]; and Peppel and Beutler, J. Cell. Biochem. Supplement 15F, 1991, p. 118 (P 439)].

B. Therapeutic and Non-therapeutic Uses for Apo-2

Apo-2, as disclosed in the present specification, can be employed therapeutically to induce apoptosis in mammalian cells. This therapy can be accomplished for instance, using *in vivo* or *ex vivo* gene therapy techniques and includes the use of the death domain sequences disclosed herein. The Apo-2 chimeric molecules (including the chimeric molecules containing the extracellular domain sequence of Apo-2) comprising immunoglobulin sequences can also be employed therapeutically to inhibit apoptosis or NF-κB induction by Apo-2L or by another ligand that Apo-2 binds to.

The Apo-2 of the invention also has utility in non-therapeutic applications. Nucleic acid sequences encoding the Apo-2 may be used as a diagnostic for tissue-specific typing. For example, procedures like *in situ* hybridization, Northern and Southern blotting, and PCR analysis may be used to determine whether DNA and/or RNA encoding Apo-2 is present in the cell type(s) being evaluated. Apo-2 nucleic acid will also be useful for the preparation of Apo-2 by the recombinant techniques described herein.

The isolated Apo-2 may be used in quantitative diagnostic assays as a control against which samples containing unknown quantities of Apo-2 may be prepared. Apo-2 preparations are also useful in generating antibodies, as standards in assays for Apo-2 (*e.g.*, by labeling Apo-2 for use as a standard in a radioimmunoassay, radioreceptor assay, or enzyme-linked immunoassay), in affinity purification techniques, and in competitive-type receptor binding assays when labeled with, for instance, radioiodine, enzymes, or fluorophores.

Modified forms of the Apo-2, such as the Apo-2-IgG chimeric molecules (immunoadhesins) described above, can be used as immunogens in producing anti-Apo-2 antibodies.

Nucleic acids which encode Apo-2 or its modified forms can also be used to generate either transgenic animals or "knock out" animals which, in turn, are useful in the development and screening of therapeutically useful reagents. A transgenic animal

(e.g., a mouse or rat) is an animal having cells that contain a transgene, which transgene was introduced into the animal or an ancestor of the animal at a prenatal, e.g., an embryonic stage. A transgene is a DNA which is integrated into the genome of a cell from which a transgenic animal develops. In one embodiment, cDNA encoding Apo-2 or an appropriate sequence thereof (such as Apo-2-IgG) can be used to clone genomic DNA encoding Apo-2 in accordance with established techniques and the genomic sequences used to generate transgenic animals that contain cells which express DNA encoding Apo-2. Methods for generating transgenic animals, particularly animals such as mice or rats, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009. Typically, particular cells would be targeted for Apo-2 transgene incorporation with tissue-specific enhancers. Transgenic animals that include a copy of a transgene encoding Apo-2 introduced into the germ line of the animal at an embryonic stage can be used to examine the effect of increased expression of DNA encoding Apo-2. Such animals can be used as tester animals for reagents thought to confer protection from, for example, pathological conditions associated with excessive apoptosis. In accordance with this facet of the invention, an animal is treated with the reagent and a reduced incidence of the pathological condition, compared to untreated animals bearing the transgene, would indicate a potential therapeutic intervention for the pathological condition. In another embodiment, transgenic animals that carry a soluble form of Apo-2 such as the Apo-2 ECD or an immunoglobulin chimera of such form could be constructed to test the effect of chronic neutralization of Apo-2L, a ligand of Apo-2.

Alternatively, non-human homologues of Apo-2 can be used to construct an Apo-2 "knock out" animal which has a defective or altered gene encoding Apo-2 as a result of homologous recombination between the endogenous gene encoding Apo-2 and altered genomic DNA encoding Apo-2 introduced into an embryonic cell of the animal. For example, cDNA encoding Apo-2 can be used to clone genomic DNA encoding Apo-2 in accordance with established techniques. A

portion of the genomic DNA encoding Apo-2 can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector [see e.g., Thomas and Capecchi, Cell, 51:503 (1987) for a description of homologous recombination vectors]. The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected [see e.g., Li et al., Cell, 69:915 (1992)]. The selected cells are then injected into a blastocyst of an animal (e.g., a mouse or rat) to form aggregation chimeras [see e.g., Bradley, in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock out" animal. Progeny harboring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized for instance, for their ability to defend against certain pathological conditions and for their development of pathological conditions due to absence of the Apo-2 polypeptide, including for example, development of tumors.

C. Anti-Apo-2 Antibody Preparation

The present invention further provides anti-Apo-2 antibodies. Antibodies against Apo-2 may be prepared as follows. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies.

1. Polyclonal Antibodies

The Apo-2 antibodies may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or

adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the Apo-2 polypeptide or a fusion protein thereof. An example of a suitable immunizing agent is a Apo-2-IgG fusion protein or chimeric molecule (including an Apo-2 ECD-IgG fusion protein). Cells expressing Apo-2 at their surface may also be employed. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins which may be employed include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. An aggregating agent such as alum may also be employed to enhance the mammal's immune response. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation. The mammal can then be bled, and the serum assayed for antibody titer. If desired, the mammal can be boosted until the antibody titer increases or plateaus.

2. Monoclonal Antibodies

The Apo-2 antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, supra. In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized (such as described above) with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

The immunizing agent will typically include the Apo-2 polypeptide or a fusion protein thereof. An example of a suitable immunizing agent is a Apo-2-IgG fusion protein or chimeric molecule. Cells expressing Apo-2 at their surface may also be employed. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or

lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103]. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Rockville, Maryland. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63].

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against Apo-2. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are

known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods [Goding, supra]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; Morrison et al., supra] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody

of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art. For instance, digestion can be performed using papain. Examples of papain digestion are described in WO 94/29348 published 12/22/94 and U.S. Patent No. 4,342,566. Papain digestion of antibodies typically produces two identical antigen binding fragments, called Fab fragments, each with a single antigen binding site, and a residual Fc fragment. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen combining sites and is still capable of cross-linking antigen.

The Fab fragments produced in the antibody digestion also contain the constant domains of the light chain and the first constant domain (CH_1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH_1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

3. Humanized Antibodies

The Apo-2 antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric

immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones et al., Nature, 321:522-525 (1986); Reichmann et al., Nature, 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567),

wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important in order to reduce antigenicity. According to the "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody [Sims et al., J. Immunol., 151:2296 (1993); Chothia and Lesk, J. Mol. Biol., 196:901 (1987)]. Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies [Carter et al., Proc. Natl. Acad. Sci. USA, 89:4285 (1992); Presta et al., J. Immunol., 151:2623 (1993)].

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three dimensional models of the parental and humanized sequences. Three dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can

be selected and combined from the consensus and import sequence so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding [see, WO 94/04679 published 3 March 1994].

Transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production can be employed. For example, it has been described that the homozygous deletion of the antibody heavy chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge [see, e.g., Jakobovits et al., Proc. Natl. Acad. Sci. USA, 90:2551-255 (1993); Jakobovits et al., Nature, 362:255-258 (1993); Bruggermann et al., Year in Immuno., 7:33 (1993)]. Human antibodies can also be produced in phage display libraries [Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)]. The techniques of Cote et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cote et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985) and Boerner et al., J. Immunol., 147(1):86-95 (1991)].

4. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the Apo-2, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have

different specificities [Millstein and Cuello, Nature, 305:537-539 (1983)]. Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker et al., EMBO J., 10:3655-3659 (1991).

According to a different and more preferred approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance. In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy-chain/light-chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in

only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690 published 3 March 1994. For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 121:210 (1986).

5. Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells [US Patent No. 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/200373; EP 03089]. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Pat. No. 4,676,980.

D. Therapeutic and Non-therapeutic Uses for Apo-2 Antibodies

The Apo-2 antibodies of the invention have therapeutic utility. Agonistic Apo-2 antibodies, for instance, may be employed to activate or stimulate apoptosis in cancer cells. Alternatively, antagonistic antibodies may be used to block excessive apoptosis (for instance in neurodegenerative disease) or to block potential autoimmune/inflammatory effects of Apo-2 resulting from NF-κB activation.

Apo-2 antibodies may further be used in diagnostic assays for Apo-2, *e.g.*, detecting its expression in specific cells, tissues, or serum. Various diagnostic assay techniques known in the art may be used, such as competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays conducted in either heterogeneous or homogeneous phases [Zola, Monoclonal Antibodies: A Manual of Techniques, CRC Press, Inc. (1987) pp. 147-158]. The antibodies used in the diagnostic assays can be labeled

with a detectable moiety. The detectable moiety should be capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase. Any method known in the art for conjugating the antibody to the detectable moiety may be employed, including those methods described by Hunter et al., Nature, 144:945 (1962); David et al., Biochemistry, 13:1014 (1974); Pain et al., J. Immunol. Meth., 40:219 (1981); and Nygren, J. Histochem. and Cytochem., 30:407 (1982).

Apo-2 antibodies also are useful for the affinity purification of Apo-2 from recombinant cell culture or natural sources. In this process, the antibodies against Apo-2 are immobilized on a suitable support, such a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody then is contacted with a sample containing the Apo-2 to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the Apo-2, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent that will release the Apo-2 from the antibody.

E. Kits Containing Apo-2 or Apo-2 Antibodies

In a further embodiment of the invention, there are provided articles of manufacture and kits containing Apo-2 or Apo-2 antibodies which can be used, for instance, for the therapeutic or non-therapeutic applications described above. The article of manufacture comprises a container with a label. Suitable containers include, for example, bottles, vials, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which includes an active agent that is effective for therapeutic or non-therapeutic applications, such as described above. The active agent in the composition is Apo-2 or an Apo-2 antibody. The label

on the container indicates that the composition is used for a specific therapy or non-therapeutic application, and may also indicate directions for either *in vivo* or *in vitro* use, such as those described above.

The kit of the invention will typically comprise the container described above and one or more other containers comprising materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

****

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

EXAMPLES

All restriction enzymes referred to in the examples were purchased from New England Biolabs and used according to manufacturer's instructions. All other commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection, Rockville, Maryland.

EXAMPLE 1

Isolation of cDNA clones Encoding Human Apo-2

Expressed sequence tag (EST) DNA databases (LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA) were searched and an EST was identified which showed homology to the death domain of the Apo-3 receptor [Marsters et al., Curr. Biol., 6:750 (1996)]. Human pancreas and kidney lgt10 bacteriophage cDNA libraries (both purchased from Clontech) were ligated into pRK5 vectors as follows.

Reagents were added together and incubated at 16°C for 16 hours: 5X T4 ligase buffer (3 ml); pRK5, Xho1, Not1 digested vector, 0.5 mg, 1 ml); cDNA (5 ml) and distilled water (6 ml). Subsequently, additional distilled water (70 ml) and 10 mg/ml tRNA (0.1 ml) were added and the entire reaction was extracted through phenol:chloroform:isoamyl alcohol (25:24:1). The aqueous phase was removed, collected and diluted into 5M NaCl (10 ml) and absolute ethanol (-20°C, 250 ml). This was then centrifuged for 20 minutes at 14,000 x g, decanted, and the pellet resuspended into 70% ethanol (0.5 ml) and centrifuged again for 2 minutes at 14,000 x g. The DNA pellet was then dried in a speedvac and eluted into distilled water (3 ml) for use in the subsequent procedure.

The ligated cDNA/pRK5 vector DNA prepared previously was chilled on ice to which was added electrocompetent DH10B bacteria (Life Tech., 20 ml). The bacteria vector mixture was then electroporated as per the manufacturers recommendation. Subsequently SOC media (1 ml) was added and the mixture was incubated at 37°C for 30 minutes. The transformants were then plated onto 20 standard 150 mm LB plates containing ampicillin and incubated for 16 hours (37°C) to allow the colonies to grow. Positive colonies were then scraped off and the DNA isolated from the bacterial pellet using standard CsCl-gradient protocols.

An enriched 5'-cDNA library was then constructed to obtain a bias of cDNA fragments which preferentially represents the 5' ends of cDNA's contained within the library. 10 mg of the pooled isolated full-length library plasmid DNA (41 ml) was combined with Not 1 restriction buffer (New England Biolabs, 5 ml) and Not 1 (New England Biolabs, 4 ml) and incubated at 37°C for one hour. The reaction was extracted through phenol:chloroform:isoamyl alcohol (25:24:1, 50 ml), the aqueous phase removed, collected and resuspended into 5M NaCl (5 ml) and absolute ethanol (-20°C, 150 ml). This was then centrifuged for 20 minutes at 14,000 x g, decanted, resuspended into 70% ethanol (0.5 ml) and centrifuged again for 2 minutes at 14,000 x g. The supernatant was then removed, the pellet dried in a speedvac and resuspended in

distilled water (10 ml).

The following reagents were brought together and incubated at 37°C for 2 hours: distilled water (3 ml); linearized DNA library (1 mg, 1 ml); Ribonucleotide mix (Invitrogen, 10 ml); transcription buffer (Invitrogen, 2 ml) and Sp6 enzyme mix. The reaction was then extracted through phenol:chloroform:isoamyl alcohol (25:24:1, 50 ml) and the aqueous phase was removed, collected and resuspended into 5M NaCl (5 ml) and absolute ethanol (-20°C, 150 ml) and centrifuged for 20 minutes at 14,000 x g. The pellet was then decanted and resuspended in 70% ethanol (0.5 ml), centrifuged again for 2 minutes at 14,000 x g, decanted, dried in a speedvac and resuspended into distilled water (10 ml).

The following reagents were added together and incubated at 16°C for 16 hours: 5X T4 ligase buffer (Life Tech., 3 ml); pRK5 Cla-Sal digested vector, 0.5 mg, 1 ml); cDNA (5 ml); distilled water (6 ml). Subsequently, additional distilled water (70 ml) and 10 mg/ml tRNA (0.1 ml) was added and the entire reaction was extracted through phenol:chloroform:isoamyl alcohol (25:24:1, 100 ml). The aqueous phase was removed, collected and diluted by 5M NaCl (10 ml) and absolute ethanol (-20°C, 250 ml) and centrifuged for 20 minutes at 14,000 x g. The DNA pellet was decanted, resuspended into 70% ethanol (0.5 ml) and centrifuged again for 2 minutes at 14,000 x g. The supernatant was removed and the residue pellet was dried in a speedvac and resuspended in distilled water (3ml). The ligated cDNA/pSST-amy.1 vector DNA was chilled on ice to which was added electrocompetent DH10B bacteria (Life Tech., 20 ml). The bacteria vector mixture was then electroporated as recommended by the manufacturer. Subsequently, SOC media (Life Tech., 1 ml) was added and the mixture was incubated at 37°C for 30 minutes. The transformants were then plated onto 20 standard 150 mm LB plates containing ampicillin and incubated for 16 hours (37°C). Positive colonies were scraped off the plates and the DNA was isolated from the bacterial pellet using standard protocols, e.g. CsCl-gradient.

The cDNA libraries were screened by hybridization with a

synthetic oligonucleotide probe:
GGGAGCCGCTCATGAGGAAGTTGGGCCTCATGGACAATGAGATAAAGGTGGCTAAAGCTGAGGCA
GCGGG (SEQ ID NO:3) based on the EST.

Three cDNA clones were sequenced in entirety. The overlapping coding regions of the cDNAs were identical except for codon 410 (using the numbering system for Fig. 1); this position encoded a leucine residue (TTG) in both pancreatic cDNAs, and a methionine residue (ATG) in the kidney cDNA, possibly due to polymorphism.

The entire nucleotide sequence of Apo-2 is shown in Figure 1 (SEQ ID NO:2). Clone 27868 (also referred to as pRK5-Apo-2 deposited as ATCC _____, as indicated below) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 140-142 [Kozak et al., supra] and ending at the stop codon found at nucleotide positions 1373-1375 (Fig. 1; SEQ ID NO:2). The predicted polypeptide precursor is 411 amino acids long, a type I transmembrane protein, and has a calculated molecular weight of approximately 45 kDa. Hydropathy analysis (not shown) suggested the presence of a signal sequence (residues 1-53), followed by an extracellular domain (residues 54-182), a transmembrane domain (residues 183-208), and an intracellular domain (residues 209-411) (Fig. 2A; SEQ ID NO:1). N-terminal amino acid sequence analysis of Apo-2-IgG expressed in 293 cells showed that the mature polypeptide starts at amino acid residue 54, indicating that the actual signal sequence comprises residues 1-53.

TNF receptor family proteins are typically characterized by the presence of multiple (usually four) cysteine-rich domains in their extracellular regions -- each cysteine-rich domain being approximately 45 amino acids long and containing approximately 6, regularly spaced, cysteine residues. Based on the crystal structure of the type 1 TNF receptor, the cysteines in each domain typically form three disulfide bonds in which usually cysteines 1 and 2, 3 and 5, and 4 and 6 are paired together. Like DR4, Apo-2 contains two extracellular cysteine-rich pseudorepeats (Fig. 2A), whereas other identified mammalian TNFR family members contain

three or more such domains [Smith et al., Cell, 76:959 (1994)].

The cytoplasmic region of Apo-2 contains a death domain (amino acid residues 324-391 shown in Fig. 1; see also Fig. 2A) which shows significantly more amino acid sequence identity to the death domain of DR4 (64%) than to the death domain of TNFR1 (30%); CD95 (19%); or Apo-3/DR3 (29%) (Fig. 2B). Four out of six death domain amino acids that are required for signaling by TNFR1 [Tartaglia et al., supra] are conserved in Apo-2 while the other two residues are semi-conserved (see Fig. 2B).

Based on an alignment analysis (using the ALIGN™ computer program) of the full-length sequence, Apo-2 shows more sequence identity to DR4 (55%) than to other apoptosis-linked receptors, such as TNFR1 (19%); CD95 (17%); or Apo-3 (also referred to as DR3, WSL-1 or TRAMP) (29%).

EXAMPLE 2

A. Expression of Apo-2 ECD

A soluble extracellular domain (ECD) fusion construct was prepared. An Apo-2 ECD (amino acid residues 1-184 shown in Figure 1) was obtained by PCR and fused to a C-terminal Flag epitope tag (Sigma). (The Apo-2 ECD construct included residues 183 and 184 shown in Figure 1 to provide flexibility at the junction, even though residues 183 and 184 are predicted to be in the transmembrane region). The Flag epitope-tagged molecule was then inserted into pRK5, and expressed by transient transfection into human 293 cells (ATCC CRL 1573).

After a 48 hour incubation, the cell supernatants were collected and either used directly for co-precipitation studies (see Example 3) or subjected to purification of the Apo-2 ECD-Flag by affinity chromatography on anti-Flag agarose beads, according to manufacturer's instructions (Sigma).

B. Expression of Apo-2 ECD as an Immunoadhesin

A soluble Apo-2 ECD immunoadhesin construct was prepared. The Apo-2 ECD (amino acids 1-184 shown in Fig. 1) was fused to the

hinge and Fc region of human immunoglobulin G_1 heavy chain in pRK5 as described previously [Ashkenazi et al., Proc. Natl. Acad. Sci., 88:10535-10539 (1991)]. The immunoadhesin was expressed by transient transfection into human 293 cells and purified from cell supernatants by protein A affinity chromatography, as described by Ashkenazi et al., supra.

EXAMPLE 3

Immunoprecipitation Assay Showing Binding Interaction Between Apo-2 and Apo-2 Ligand

To determine whether Apo-2 and Apo-2L interact or associate with each other, supernatants from mock-transfected 293 cells or from 293 cells transfected with Apo-2 ECD-Flag (described in Example 2 above) (5 ml) were incubated with 5 μg poly-histidine-tagged soluble Apo-2L [Pitti et al., supra] for 30 minutes at room temperature and then analyzed for complex formation by a co-precipitation assay.

The samples were subjected to immunoprecipitation using 25 μl anti-Flag conjugated agarose beads (Sigma) or Nickel-conjugated agarose beads (Qiagen). After a 1.5 hour incubation at 4° C, the beads were spun down and washed four times in phosphate buffered saline (PBS). By using anti-Flag agarose, the Apo-2L was precipitated through the Flag-tagged Apo-2 ECD; by using Nickel-agarose, the Apo-2 ECD was precipitated through the His-tagged Apo-2L. The precipitated proteins were released by boiling the beads for 5 minutes in SDS-PAGE buffer, resolved by electrophoresis on 12% polyacrylamide gels, and then detected by immunoblot with anti-Apo-2L or anti-Flag antibody (2 μg/ml) as described in Marsters et al., J. Biol. Chem., (1997).

The results, shown in Figure 3, indicate that the Apo-2 ECD and Apo-2L can associate with each other.

The binding interaction was further analyzed by purifying Apo-2 ECD from the transfected 293 cell supernatants with anti-Flag beads (see Example 2) and then analyzing the samples on a BIACORE™ instrument. The BIACORE™ analysis indicated a dissociation

constant (K_d) of about 1 nM. BIACORE™ analysis also showed that the Apo-2 ECD is not capable of binding other apoptosis-inducing TNF family members, namely, TNF-alpha (Genentech, Inc., Pennica et al., Nature, 312:712 (1984), lymphotoxin-alpha (Genentech, Inc.), or Fas/Apo-1 ligand (Alexis Biochemicals). The data thus shows that Apo-2 is a specific receptor for Apo-2L.

EXAMPLE 4

Induction of Apoptosis by Apo-2

Because death domains can function as oligomerization interfaces, over-expression of receptors that contain death domains may lead to activation of signaling in the absence of ligand [Frazer et al., supra, Nagata et al., supra]. To determine whether Apo-2 was capable of inducing cell death, human 293 cells or HeLa cells (ATCC CCL 2.2) were transiently transfected by calcium phosphate precipitation (293 cells) or electroporation (HeLa cells) with a pRK5 vector or pRK5-based plasmids encoding Apo-2 and/or CrmA. When applicable, the total amount of plasmid DNA was adjusted by adding vector DNA. Apoptosis was assessed 24 hours after transfection by morphology (Fig. 4A); DNA fragmentation (Fig. 4B); or by FACS analysis of phosphatydilserine exposure (Fig. 4C) as described in Marsters et al., Curr. Biol., 6:1669 (1996). As shown in Figs. 4A and 4B, the Apo-2 transfected 293 cells underwent marked apoptosis.

For samples assayed by FACS, the HeLa cells were co-transfected with pRK5-CD4 as a marker for transfection and apoptosis was determined in CD4-expressing cells; FADD was co-transfected with the Apo-2 plasmid; the data are means ± SEM of at least three experiments, as described in Marsters et al., Curr. Biol., 6:1669 (1996). The caspase inhibitors, DEVD-fmk (Enzyme Systems) or z-VAD-fmk (Research Biochemicals Intl.) were added at 200 μM at the time of transfection. As shown in Fig. 4C, the caspase inhibitors CrmA, DEVD-fmk, and z-VAD-fmk blocked apoptosis induction by Apo-2, indicating the involvement of Ced-3-like proteases in this response.

FADD is an adaptor protein that mediates apoptosis activation by CD95, TNFR1, and Apo-3/DR3 [Nagata et al., supra], but does not appear necessary for apoptosis induction by Apo-2L [Marsters et al., supra] or by DR4 [Pan et al., supra]. A dominant-negative mutant form of FADD, which blocks apoptosis induction by CD95, TNFR1, or Apo-3/DR3 [Frazer et al., supra; Nagata et al., supra; Chinnayian et al., supra] did not inhibit apoptosis induction by Apo-2 when co-transfected into HeLa cells with Apo-2 (Fig. 4C). These results suggest that Apo-2 signals apoptosis independently of FADD. Consistent with this conclusion, a glutathione-S-transferase fusion protein containing the Apo-2 cytoplasmic region did not bind to *in vitro* transcribed and translated FADD (data not shown).

EXAMPLE 5

Inhibition of Apo-2L Activity by Soluble Apo-2 ECD

Soluble Apo-2L (0.5 μg/ml, prepared as described in Pitti et al., supra) was pre-incubated for 1 hour at room temperature with PBS buffer or affinity-purified Apo-2 ECD (5 μg/ml) together with anti-Flag antibody (Sigma) (1 μg/ml) and added to HeLa cells. After a 5 hour incubation, the cells were analyzed for apoptosis by FACS (as above) (Fig. 4D).

Apo-2L induced marked apoptosis in HeLa cells, and the soluble Apo-2 ECD was capable of blocking Apo-2L action (Fig. 4D), confirming a specific interaction between Apo-2L and Apo-2. Similar results were obtained with the Apo-2 ECD immunoadhesin (Fig. 4D). Dose-response analysis showed half-maximal inhibition at approximately 0.3 nM Apo-2 immunoadhesin (Fig. 4E).

EXAMPLE 6

Activation of NF-κB by Apo-2

An assay was conducted to determine whether Apo-2 activates NF-κB.

HeLa cells were transfected with pRK5 expression plasmids encoding full-length native sequence Apo-2, DR4 or Apo-3 and

harvested 24 hours after transfection. Nuclear extracts were prepared and 1 μg of nuclear protein was reacted with a ^{32}P-labelled NF-κB-specific synthetic oligonucleotide probe ATCAGGGACTTTCCGCTGGGGACTTTCCG (SEQ ID NO:4) [see, also, MacKay et al., J. Immunol., 153:5274-5284 (1994)], alone or together with a 50-fold excess of unlabelled probe, or with an irrelevant ^{32}P-labelled synthetic oligonucleotide AGGATGGGAAGTGTGTGATATATCCTTGAT (SEQ ID NO:5). In some samples, antibody to p65/RelA subunits of NF-κB (1 μg/ml; Santa Cruz Biotechnology) was added. DNA binding was analyzed by an electrophoretic mobility shift assay as described by Hsu et al., supra; Marsters et al., supra, and MacKay et al., supra.

The results are shown in Fig. 5. As shown in Fig. 5A, upon transfection into HeLa cells, both Apo-2 and DR4 induced significant NF-κB activation as measured by the electrophoretic mobility shift assay; the level of activation was comparable to activation observed for Apo-3/DR3. Antibody to the p65/RelA subunit of NF-κB inhibited the mobility of the NF-κB probe, implicating p65 in the response to all 3 receptors.

An assay was also conducted to determine if Apo-2L itself can regulate NF-κB activity. HeLa cells or MCF7 cells (human breast adenocarcinoma cell line, ATCC HTB 22) were treated with PBS buffer, soluble Apo-2L (Pitti et al., supra) or TNF-alpha (Genentech, Inc., see Pennica et al., Nature, 312:721 (1984)) (1 μg/ml) and assayed for NF-κB activity as above. The results are shown in Fig. 5B. The Apo-2L induced a significant NF-κB activation in the treated HeLa cells but not in the treated MCF7 cells; the TNF-alpha induced a more pronounced activation in both cell lines. Several studies have disclosed that NF-κB activation by TNF can protect cells against TNF-induced apoptosis [Nagata, supra].

The effects of a NF-κB inhibitor, ALLN (N-acetyl-Leu-Leu-norleucinal) and a transcription inhibitor, cyclohexamide, were also tested. The HeLa cells (plated in 6-well dishes) were preincubated with PBS buffer, ALLN (Calbiochem) (40 μg/ml) or

cyclohexamide (Sigma) (50 μg/ml) for 1 hour before addition of Apo-2L (1 μg/ml). After a 5 hour incubation, apoptosis was analyzed by FACS (see Fig. 5C).

The results are shown in Fig. 5C. Both ALLN and cyclohexamide increased the level of Apo-2L-induced apoptosis in the HeLa cells. The data indicates that Apo-2L can induce protective NF-κB-dependent genes. The data also indicates that Apo-2L is capable of activating NF-κB in certain cell lines and that both Apo-2 and DR4 may mediate that function.

EXAMPLE 7

Northern Blot Analysis

Expression of Apo-2 mRNA in human tissues was examined by Northern blot analysis. Human RNA blots were hybridized to a 4.6 kilobase ^{32}P-labelled DNA probe based on the full length Apo-2 cDNA; the probe was generated by digesting the pRK5-Apo-2 plasmid with EcoRI. Human fetal RNA blot MTN (Clontech) and human adult RNA blot MTN-II (Clontech) were incubated with the DNA probes. Blots were incubated with the probes in hybridization buffer (5X SSPE; 2X Denhardt's solution; 100 mg/mL denatured sheared salmon sperm DNA; 50% formamide; 2% SDS) for 60 hours at 42°C. The blots were washed several times in 2X SSC; 0.05% SDS for 1 hour at room temperature, followed by a 30 minute wash in 0.1X SSC; 0.1% SDS at 50°C. The blots were developed after overnight exposure.

As shown in Fig. 6, a predominant mRNA transcript of approximately 4.6kb was detected in multiple tissues. Expression was relatively high in fetal and adult liver and lung, and in adult ovary and peripheral blood leukocytes (PBL), while no mRNA expression was detected in fetal and adult brain. Intermediate levels of expression were seen in adult colon, small intestine, testis, prostate, thymus, pancreas, lidney, skeletal muscle, placenta, and heart. Several adult tissues that express Apo-2, e.g., PBL, ovary, and spleen, have been shown previously to express DR4 [Pan et al., supra], however, the relative levels of expression of each receptor mRNA appear to be different.

EXAMPLE 8

Chromosomal Localization of the Apo-2 gene

Chromosomal localization of the human Apo-2 gene was examined by radiation hybrid (RH) panel analysis. RH mapping was performed by PCR using a human-mouse cell radiation hybrid panel (Research Genetics) and primers based on the coding region of the Apo-2 cDNA [Gelb et al., Hum. Genet., 98:141 (1996)]. Analysis of the PCR data using the Stanford Human Genome Center Database indicates that Apo-2 is linked to the marker D8S481, with an LOD of 11.05; D8S481 is linked in turn to D8S2055, which maps to human chromosome 8p21. A similar analysis of DR4 showed that DR4 is linked to the marker D8S2127 (with an LOD of 13.00), which maps also to human chromosome 8p21.

To Applicants' present knowledge, to date, no other member of the TNFR gene family has been located to chromosome 8.

* * * * *

Deposit of Material

The following materials have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, USA (ATCC):

Material	ATCC Dep. No.	Deposit Date
pRK5-Apo-2	_____	_____

This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the

invention. The present invention is not to be limited in scope by the construct deposited, since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Ashkenazi, Avi J.

(ii) TITLE OF INVENTION: Apo-2 RECEPTOR

(iii) NUMBER OF SEQUENCES: 5

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Genentech, Inc.
(B) STREET: 460 Point San Bruno Blvd
(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: WinPatin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 15-May-1997
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Marschang, Diane L.
(B) REGISTRATION NUMBER: 35,600
(C) REFERENCE/DOCKET NUMBER: P1101

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 415/225-5416
(B) TELEFAX: 415/952-9881
(C) TELEX: 910/371-7168

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 411 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Glu Gln Arg Gly Gln Asn Ala Pro Ala Ala Ser Gly Ala Arg
1 5 10 15

Lys Arg His Gly Pro Gly Pro Arg Glu Ala Arg Gly Ala Arg Pro
20 25 30

Gly Leu Arg Val Pro Lys Thr Leu Val Leu Val Val Ala Ala Val
35 40 45

Leu Leu Leu Val Ser Ala Glu Ser Ala Leu Ile Thr Gln Gln Asp
50 55 60

Leu Ala Pro Gln Gln Arg Ala Ala Pro Gln Gln Lys Arg Ser Ser
65 70 75

Pro Ser Glu Gly Leu Cys Pro Pro Gly His His Ile Ser Glu Asp
80 85 90

Gly Arg Asp Cys Ile Ser Cys Lys Tyr Gly Gln Asp Tyr Ser Thr
95 100 105

His Trp Asn Asp Leu Leu Phe Cys Leu Arg Cys Thr Arg Cys Asp

110 115 120

Ser Gly Glu Val Glu Leu Ser Pro Cys Thr Thr Thr Arg Asn Thr
125 130 135

Val Cys Gln Cys Glu Glu Gly Thr Phe Arg Glu Glu Asp Ser Pro
140 145 150

Glu Met Cys Arg Lys Cys Arg Thr Gly Cys Pro Arg Gly Met Val
155 160 165

Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile Glu Cys Val His
170 175 180

Lys Glu Ser Gly Ile Ile Ile Gly Val Thr Val Ala Ala Val Val
185 190 195

Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp Lys Lys
200 205 210

Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly Asp
215 220 225

Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp
230 235 240

Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val
245 250 255

Pro Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly
260 265 270

Val Asn Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu Pro
275 280 285

Ala Glu Ala Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala
290 295 300

Asn Glu Gly Asp Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp
305 310 315

Phe Ala Asp Leu Val Pro Phe Asp Ser Trp Glu Pro Leu Met Arg
320 325 330

Lys Leu Gly Leu Met Asp Asn Glu Ile Lys Val Ala Lys Ala Glu
335 340 345

Ala Ala Gly His Arg Asp Thr Leu Tyr Thr Met Leu Ile Lys Trp
350 355 360

Val Asn Lys Thr Gly Arg Asp Ala Ser Val His Thr Leu Leu Asp
365 370 375

Ala Leu Glu Thr Leu Gly Glu Arg Leu Ala Lys Gln Lys Ile Glu
380 385 390

Asp His Leu Leu Ser Ser Gly Lys Phe Met Tyr Leu Glu Gly Asn
395 400 405

Ala Asp Ser Ala Xaa Ser
410 411

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1799 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
CCCACGCGTC CGCATAAATC AGCACGCGGC CGGAGAACCC CGCAATCTCT 50

GCGCCCACAA AATACACCGA CGATGCCCGA TCTACTTTAA GGGCTGAAAC 100

CCACGGGCCT GAGAGACTAT AAGAGCGTTC CCTACCGCC  ATG GAA 145
                                           Met Glu
                                             1

CAA CGG GGA CAG AAC GCC CCG GCC GCT TCG GGG GCC CGG 184
Gln Arg Gly Gln Asn Ala Pro Ala Ala Ser Gly Ala Arg
          5                  10                  15

AAA AGG CAC GGC CCA GGA CCC AGG GAG GCG CGG GGA GCC 223
Lys Arg His Gly Pro Gly Pro Arg Glu Ala Arg Gly Ala
                 20                  25

AGG CCT GGG CTC CGG GTC CCC AAG ACC CTT GTG CTC GTT 262
Arg Pro Gly Leu Arg Val Pro Lys Thr Leu Val Leu Val
     30                  35                  40

GTC GCC GCG GTC CTG CTG TTG GTC TCA GCT GAG TCT GCT 301
Val Ala Ala Val Leu Leu Leu Val Ser Ala Glu Ser Ala
               45                  50

CTG ATC ACC CAA CAA GAC CTA GCT CCC CAG CAG AGA GCG 340
Leu Ile Thr Gln Gln Asp Leu Ala Pro Gln Gln Arg Ala
 55                  60                  65

GCC CCA CAA CAA AAG AGG TCC AGC CCC TCA GAG GGA TTG 379
Ala Pro Gln Gln Lys Arg Ser Ser Pro Ser Glu Gly Leu
          70                  75                  80
```

```
TGT CCA CCT GGA CAC CAT ATC TCA GAA GAC GGT AGA GAT 418
Cys Pro Pro Gly His His Ile Ser Glu Asp Gly Arg Asp
                 85                  90

TGC ATC TCC TGC AAA TAT GGA CAG GAC TAT AGC ACT CAC 457
Cys Ile Ser Cys Lys Tyr Gly Gln Asp Tyr Ser Thr His
     95                 100                 105

TGG AAT GAC CTC CTT TTC TGC TTG CGC TGC ACC AGG TGT 496
Trp Asn Asp Leu Leu Phe Cys Leu Arg Cys Thr Arg Cys
            110                 115

GAT TCA GGT GAA GTG GAG CTA AGT CCC TGC ACC ACG ACC 535
Asp Ser Gly Glu Val Glu Leu Ser Pro Cys Thr Thr Thr
120                 125                 130

AGA AAC ACA GTG TGT CAG TGC GAA GAA GGC ACC TTC CGG 574
Arg Asn Thr Val Cys Gln Cys Glu Glu Gly Thr Phe Arg
        135                 140                 145

GAA GAA GAT TCT CCT GAG ATG TGC CGG AAG TGC CGC ACA 613
Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys Arg Thr
                150                 155

GGG TGT CCC AGA GGG ATG GTC AAG GTC GGT GAT TGT ACA 652
Gly Cys Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr
    160                 165                 170

CCC TGG AGT GAC ATC GAA TGT GTC CAC AAA GAA TCA GGC 691
Pro Trp Ser Asp Ile Glu Cys Val His Lys Glu Ser Gly
            175                 180

ATC ATC ATA GGA GTC ACA GTT GCA GCC GTA GTC TTG ATT 730
Ile Ile Ile Gly Val Thr Val Ala Ala Val Val Leu Ile
185                 190                 195
```

```
GTG GCT GTG TTT GTT TGC AAG TCT TTA CTG TGG AAG AAA 769
Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp Lys Lys
        200                 205                 210

GTC CTT CCT TAC CTG AAA GGC ATC TGC TCA GGT GGT GGT 808
Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly
                215                 220

GGG GAC CCT GAG CGT GTG GAC AGA AGC TCA CAA CGA CCT 847
Gly Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro
    225                 230                 235

GGG GCT GAG GAC AAT GTC CTC AAT GAG ATC GTG AGT ATC 886
Gly Ala Glu Asp Asn Val Leu Asn Glu Ile Val Ser Ile
            240                 245

TTG CAG CCC ACC CAG GTC CCT GAG CAG GAA ATG GAA GTC 925
Leu Gln Pro Thr Gln Val Pro Glu Gln Glu Met Glu Val
250                 255                 260

CAG GAG CCA GCA GAG CCA ACA GGT GTC AAC ATG TTG TCC 964
Gln Glu Pro Ala Glu Pro Thr Gly Val Asn Met Leu Ser
        265                 270                 275

CCC GGG GAG TCA GAG CAT CTG CTG GAA CCG GCA GAA GCT 1003
Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala Glu Ala
                280                 285

GAA AGG TCT CAG AGG AGG AGG CTG CTG GTT CCA GCA AAT 1042
Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala Asn
    290                 295                 300

GAA GGT GAT CCC ACT GAG ACT CTG AGA CAG TGC TTC GAT 1081
Glu Gly Asp Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp
            305                 310
```

```
GAC TTT GCA GAC TTG GTG CCC TTT GAC TCC TGG GAG CCG 1120
Asp Phe Ala Asp Leu Val Pro Phe Asp Ser Trp Glu Pro
315                 320                 325

CTC ATG AGG AAG TTG GGC CTC ATG GAC AAT GAG ATA AAG 1159
Leu Met Arg Lys Leu Gly Leu Met Asp Asn Glu Ile Lys
        330                 335                 340

GTG GCT AAA GCT GAG GCA GCG GGC CAC AGG GAC ACC TTG 1198
Val Ala Lys Ala Glu Ala Ala Gly His Arg Asp Thr Leu
                345                 350

TAC ACG ATG CTG ATA AAG TGG GTC AAC AAA ACC GGG CGA 1237
Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr Gly Arg
    355                 360                 365

GAT GCC TCT GTC CAC ACC CTG CTG GAT GCC TTG GAG ACG 1276
Asp Ala Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr
            370                 375

CTG GGA GAG AGA CTT GCC AAG CAG AAG ATT GAG GAC CAC 1315
Leu Gly Glu Arg Leu Ala Lys Gln Lys Ile Glu Asp His
380                 385                 390

TTG TTG AGC TCT GGA AAG TTC ATG TAT CTA GAA GGT AAT 1354
Leu Leu Ser Ser Gly Lys Phe Met Tyr Leu Glu Gly Asn
        395                 400                 405

GCA GAC TCT GCC WTG TCC TAAGTGTG ATTCTCTTCA GGAAGTGAGA 1400
Ala Asp Ser Ala Xaa Ser
                410 411

CCTTCCCTGG TTTACCTTTT TTCTGGAAAA AGCCCAACTG GACTCCAGTC 1450

AGTAGGAAAG TGCCACAATT GTCACATGAC CGGTACTGGA AGAAACTCTC 1500
```

CCATCCAACA TCACCCAGTG GATGGAACAT CCTGTAACTT TTCACTGCAC 1550

TTGGCATTAT TTTTATAAGC TGAATGTGAT AATAAGGACA CTATGGAAAT 1600

GTCTGGATCA TTCCGTTTGT GCGTACTTTG AGATTTGGTT TGGGATGTCA 1650

TTGTTTTCAC AGCACTTTTT TATCCTAATG TAAATGCTTT ATTTATTTAT 1700

TTGGGCTACA TTGTAAGATC CATCTACAAA AAAAAAAAAA AAAAAAAAAG 1750

GGCGGCCGCG ACTCTAGAGT CGACCTGCAG AAGCTTGGCC GCCATGGCC 1799

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGGAGCCGCT CATGAGGAAG TTGGGCCTCA TGGACAATGA GATAAAGGTG 50

GCTAAAGCTG AGGCAGCGGG 70

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ATCAGGGACT TTCCGCTGGG GACTTTCCG 29

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGGATGGGAA GTGTGTGATA TATCCTTGAT 30

WHAT IS CLAIMED IS:

1. A method of modulating apoptosis in mammalian cells comprising exposing mammalian cells to Apo-2 polypeptide, said Apo-2 polypeptide having at least about 80% amino acid sequence identity with native sequence Apo-2 polypeptide comprising amino acid residues 1 to 411 of SEQ ID NO:1.

2. A method of modulating apoptosis in mammalian cells comprising exposing mammalian cells to an extracellular domain sequence of Apo-2 polypeptide comprising amino acid residues 54 to 182 of SEQ ID NO:1.

Abstract of the Disclosure

Novel polypeptides, designated Apo-2, which are capable of modulating apoptosis are provided. Compositions including Apo-2 chimeras, nucleic acid encoding Apo-2, and antibodies to Apo-2 are also provided.

Fig. 1

```
   1 CCCACGCGTC CGCATAAATC AGCACGCGGC CGGAGAACCC CGCAATCTCT GCGCCCACAA AATACACCGA CGATGCCCGA TCTACTTTAA GGGCTGAAAC
     GGGTGCGCAG GCGTATTTAG TCGTGCGCCG GCCTCTTGGG GCGTTAGAGA CGCGGGTGTT TTATGTGGCT GCTACGGGCT AGATGAAATT CCCGACTTTG

 101 CCACGGGCCT GAGAGACTAT AAGAGCGTTC CCTACCGCCA TGGAACAACG GGGACAGAAC GCCCCGGCCG CTTCGGGGGC CCGGAAAAGG CACGGCCCAG
     GGTGCCCGGA CTCTCTGATA TTCTCGCAAG GGATGGCGGT ACCTTGTTGC CCCTGTCTTG CGGGGCCGGC GAAGCCCCCG GGCCTTTTCC GTGCCGGGTC
   1                                          M etGluGlnAr gGlyGlnAsn AlaProAlaA laSerGlyAl aArgLysArg HisGlyProGly

 201 GACCCAGGGA GGCGCGGGGA GCCAGGCCTG GGCTCCGGGT CCCCAAGACC CTTGTGCTCG TTGTCGCCGC GGTCCTGCTG TTGGTCTCAG CTGAGTCTGC
     CTGGGTCCCT CCGCGCCCCT CGGTCCGGAC CCGAGGCCCA GGGGTTCTGG GAACACGAGC AACAGCGGCG CCAGGACGAC AACCAGAGTC GACTCAGACG
  22   ProArgGl uAlaArgGly AlaArgProG lyLeuArgVa lProLysThr LeuValLeuV alValAlaAl aValLeuLeu LeuValSerA laGluSerAla

 301 TCTGATCACC CAACAAGACC TAGCTCCCCA GCAGAGAGCG GCCCCACAAC AAAAGAGGTC CAGCCCCTCA GAGGGATTGT GTCCACCTGG ACACCATATC
     AGACTAGTGG GTTGTTCTGG ATCGAGGGGT CGTCTCTCGC CGGGGTGTTG TTTTCTCCAG GTCGGGGAGT CTCCCTAACA CAGGTGGACC TGTGGTATAG
  55  LeuIleThr GlnGlnAspL euAlaProGl nGlnArgAla AlaProGlnG lnLysArgSe rSerProSer GluGlyLeuC ysProProGl yHisHisIle

 401 TCAGAAGACG GTAGAGATTG CATCTCCTGC AAATATGGAC AGGACTATAG CACTCACTGG AATGACCTCC TTTTCTGCTT GCGCTGCACC AGGTGTGATT
     AGTCTTCTGC CATCTCTAAC GTAGAGGACG TTTATACCTG TCCTGATATC GTGAGTGACC TTACTGGAGG AAAAGACGAA CGCGACGTGG TCCACACTAA
  88 SerGluAspG lyArgAspCy sIleSerCys LysTyrGlyG lnAspTyrSe rThrHisTrp AsnAspLeuL euPheCysLe uArgCysThr ArgCysAspSer

 501 CAGGTGAAGT GGAGCTAAGT CCCTGCACCA CGACCAGAAA CACAGTGTGT CAGTGCGAAG AAGGCACCTT CCGGGAAGAA GATTCTCCTG AGATGTGCCG
     GTCCACTTCA CCTCGATTCA GGGACGTGGT GCTGGTCTTT GTGTCACACA GTCACGCTTC TTCCGTGGAA GGCCCTTCTT CTAAGAGGAC TCTACACGGC
 122   GlyGluVa lGluLeuSer ProCysThrT hrThrArgAs nThrValCys GlnCysGluG luGlyThrPh eArgGluGlu AspSerProG luMetCysArg

 601 GAAGTGCCGC ACAGGGTGTC CCAGAGGGAT GGTCAAGGTC GGTGATTGTA CACCCTGGAG TGACATCGAA TGTGTCCACA AAGAATCAGG CATCATCATA
     CTTCACGGCG TGTCCCACAG GGTCTCCCTA CCAGTTCCAG CCACTAACAT GTGGGACCTC ACTGTAGCTT ACACAGGTGT TTCTTAGTCC GTAGTAGTAT
 155  LysCysArg ThrGlyCysP roArgGlyMe tValLysVal GlyAspCysT hrProTrpSe rAspIleGlu CysValHisL ysGluSerGl yIleIleIle

 701 GGAGTCACAG TTGCAGCCGT AGTCTTGATT GTGGCTGTGT TTGTTTGCAA GTCTTTACTG TGGAAGAAAG TCCTTCCTTA CCTGAAAGGC ATCTGCTCAG
     CCTCAGTGTC AACGTCGGCA TCAGAACTAA CACCGACACA AACAAACGTT CAGAAATGAC ACCTTCTTTC AGGAAGGAAT GGACTTTCCG TAGACGAGTC
 188 GlyValThrV alAlaAlaVa lValLeuIle ValAlaValP heValCysLy sSerLeuLeu TrpLysLysV alLeuProTy rLeuLysGly IleCysSerGly

 801 GTGGTGGTGG GGACCCTGAG CGTGTGGACA GAAGCTCACA ACGACCTGGG GCTGAGGACA ATGTCCTCAA TGAGATCGTG AGTATCTTGC AGCCCACCCA
     CACCACCACC CCTGGGACTC GCACACCTGT CTTCGAGTGT TGCTGGACCC CGACTCCTGT TACAGGAGTT ACTCTAGCAC TCATAGAACG TCGGGTGGGT
 222   GlyGlyGl yAspProGlu ArgValAspA rgSerSerGl nArgProGly AlaGluAspA snValLeuAs nGluIleVal SerIleLeuG lnProThrGln

 901 GGTCCCTGAG CAGGAAATGG AAGTCCAGGA GCCAGCAGAG CCAACAGGTG TCAACATGTT GTCCCCCGGG GAGTCAGAGC ATCTGCTGGA ACCGGCAGAA
     CCAGGGACTC GTCCTTTACC TTCAGGTCCT CGGTCGTCTC GGTTGTCCAC AGTTGTACAA CAGGGGGCCC CTCAGTCTCG TAGACGACCT TGGCCGTCTT
 255  ValProGlu GlnGluMetG luValGlnGl uProAlaGlu ProThrGlyV alAsnMetLe uSerProGly GluSerGluH isLeuLeuGl uProAlaGlu

1001 GCTGAAAGGT CTCAGAGGAG GAGGCTGCTG GTTCCAGCAA ATGAAGGTGA TCCCACTGAG ACTCTGAGAC AGTGCTTCGA TGACTTTGCA GACTTGGTGC
     CGACTTTCCA GAGTCTCCTC CTCCGACGAC CAAGGTCGTT TACTTCCACT AGGGTGACTC TGAGACTCTG TCACGAAGCT ACTGAAACGT CTGAACCACG
 288 AlaGluArgS erGlnArgAr gArgLeuLeu ValProAlaA snGluGlyAs pProThrGlu ThrLeuArgG lnCysPheAs pAspPheAla AspLeuValPro
```

```
1101 CCTTTGACTC CTGGGAGCCG CTCATGAGGA AGTTGGGCCT CATGGACAAT GAGATAAAGG TGGCTAAAGC TGAGGCAGCG GGCCACAGGG ACACCTTGTA
     GGAAACTGAG GACCCTCGGC GAGTACTCCT TCAACCCGGA GTACCTGTTA CTCTATTTCC ACCGATTTCG ACTCCGTCGC CCGGTGTCCC TGTGGAACAT
 322   PheAspSe rTrpGluPro LeuMetArgL ysLeuGlyLe uMetAspAsn GluIleLysV alAlaLysAl aGluAlaAla GlyHisArgA spThrLeuTyr

1201 CACGATGCTG ATAAAGTGGG TCAACAAAAC CGGGCGAGAT GCCTCTGTCC ACACCCTGCT GGATGCCTTG GAGACGCTGG GAGAGAGACT TGCCAAGCAG
     GTGCTACGAC TATTTCACCC AGTTGTTTTG GCCCGCTCTA CGGAGACAGG TGTGGGACGA CCTACGGAAC CTCTGCGACC CTCTCTCTGA ACGGTTCGTC
 355  ThrMetLeu IleLysTrpV alAsnLysTh rGlyArgAsp AlaSerValH isThrLeuLe uAspAlaLeu GluThrLeuG lyGluArgLe uAlaLysGln

1301 AAGATTGAGG ACCACTTGTT GAGCTCTGGA AAGTTCATGT ATCTAGAAGG TAATGCAGAC TCTGCCWTGT CCTAAGTGTG ATTCTCTTCA GGAAGTGAGA
     TTCTAACTCC TGGTGAACAA CTCGAGACCT TTCAAGTACA TAGATCTTCC ATTACGTCTG AGACGGAACA GGATTCACAC TAAGAGAAGT CCTTCACTCT
 388 LysIleGluA spHisLeuLe uSerSerGly LysPheMetT yrLeuGluGl yAsnAlaAsp SerAlaXqqS erOC*

1401 CCTTCCCTGG TTTACCTTTT TTCTGGAAAA AGCCCAACTG GACTCCAGTC AGTAGGAAAG TGCCACAATT GTCACATGAC CGGTACTGGA AGAAACTCTC
     GGAAGGGACC AAATGGAAAA AAGACCTTTT TCGGGTTGAC CTGAGGTCAG TCATCCTTTC ACGGTGTTAA CAGTGTACTG GCCATGACCT TCTTTGAGAG

  01 CCATCCAACA TCACCCAGTG GATGGAACAT CCTGTAACTT TTCACTGCAC TTGGCATTAT TTTTATAAGC TGAATGTGAT AATAAGGACA CTATGGAAAT
     GGTAGGTTGT AGTGGGTCAC CTACCTTGTA GGACATTGAA AAGTGACGTG AACCGTAATA AAAATATTCG ACTTACACTA TTATTCCTGT GATACCTTTA

1601 GTCTGGATCA TTCCGTTTGT GCGTACTTTG AGATTTGGTT TGGGATGTCA TTGTTTTCAC AGCACTTTTT TATCCTAATG TAAATGCTTT ATTTATTTAT
     CAGACCTAGT AAGGCAAACA CGCATGAAAC TCTAAACCAA ACCCTACAGT AACAAAAGTG TCGTGAAAAA ATAGGATTAC ATTTACGAAA TAAATAAATA

1701 TTGGGCTACA TTGTAAGATC CATCTACAAA AAAAAAAAAA AAAAAAAAAG GGCGGCCGCG ACTCTAGAGT CGACCTGCAG AAGCTTGGCC GCCATGGCC
     AACCCGATGT AACATTCTAG GTAGATGTTT TTTTTTTTTT TTTTTTTTTC CCGCCGGCGC TGAGATCTCA GCTGGACGTC TTCGAACCGG CGGTACCGG
```

Fig. 1 (cont.)

FIG. 2A

```
  1 MEQRGQNAPAASGARKRHGPGPREARGARPGLRVPKTLVLVVAAVLLLVSAESALITQQD
 61 LAPQQRAAPQQKRSSPSEGLCPPGHHISEDGRDCISCKYGQDYSTHWNDLLFCLRCTRCD
121 SGEVELSPCTTTRNTVCQCEEGTFREEDSPEMCRKCRTGCPRGMVKVGDCTPWSDIECVH
181 KESGIIIGVTVAAVVLIVAVFVCKSLLWKKVLPYLKGICSGGGGDPERVDRSSQRPGAED
241 NVLNEIVSILQPTQVPEQEMEVQEPAEPTGVNMLSPGESEHLLEPAEAERSQRRRLLVPA
301 NEGDPTETLRQCFDDFADLVPFDSWEPLMRKLGLMDNEIKVAKAEAAGHRDTLYTMLIKW
361 VNKTGRDASVHTLLDALETLGERLAKQKIEDHLLSSGKFMYLEGNADSALS
```

FIG. 2B

```
Apo2       FADLVPFDSWEPLMRKLGLMDNEIKVAKAEAA--GHRDTL
DR4        FANIVPFDSWDQLMRQLDLTKNEIDVVRAGTA--GPGDAL
Apo3/DR3   VMDAVPARRWKEFVRTLGLREAEIEAVEVEIGR--FRDQQ
TNFR1      VVENVPPLRWKEFVRRLGLSDHEIDRLELQNGR-CLREAQ
Fas/Apo1   IAGVMTLSQVKGFVRKNGVNEAKIDEIKNDNVQDTAEQKV

Apo2       YTMLIKWVNKTGRD-ASVHTLLDALETLGERLAKQKIED
DR4        YAMLMKWVNKTGRN-ASIHTLLDALERMEERHAKEKIQD
Apo3/DR3   YEMLKRWRQQQP---AGLGAVYAALERMGLDGCVEDLRS
TNFR1      YSMLATWRRRTPRREATLELLGRVLRDMDLLGCLEDIEE
Fas/Apo1   -QLLRNWHQLHGKKEAY-DTLIKDLKKANLCTLAEKIQT
```

Fig. 3

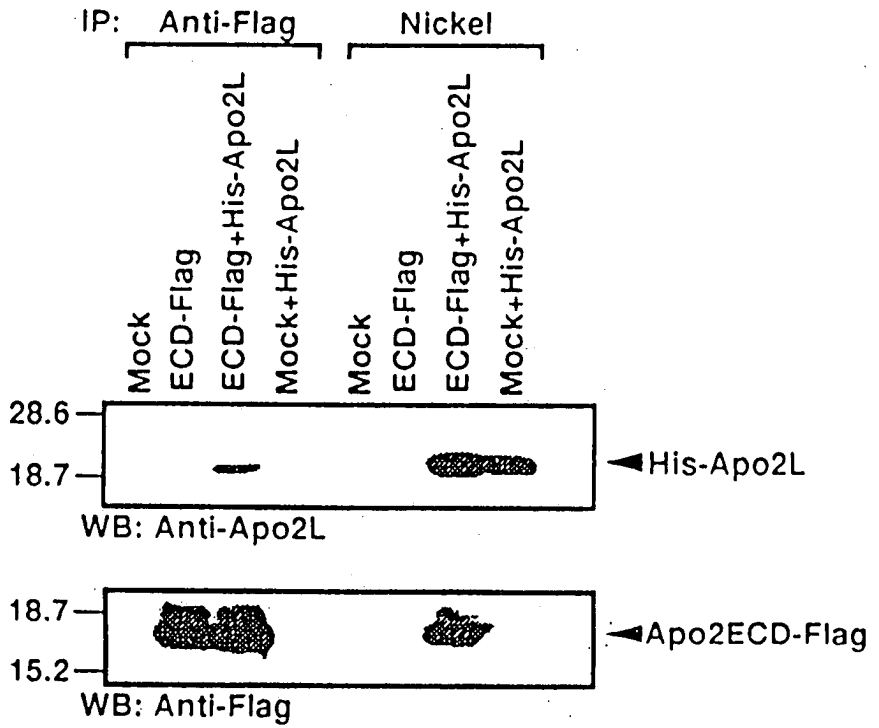
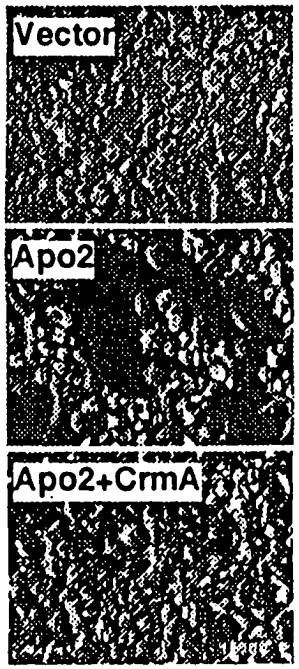
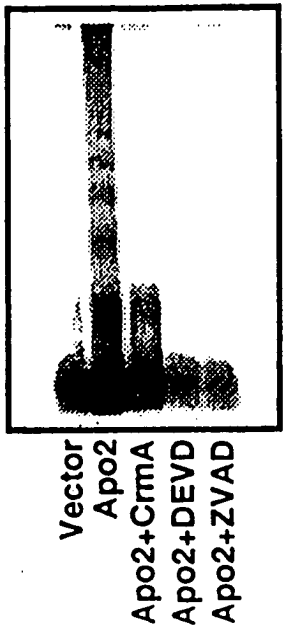

IP: Anti-Flag
Nickel
Mock
ECD-Flag
ECD-Flag+His-Apo2L
Mock+His-Apo2L
Mock
ECD-Flag
ECD-Flag+His-Apo2L
Mock+His-Apo2L
28.6
18.7
His-Apo2L
WB: Anti-Apo2L
18.7
15.2
Apo2ECD-Flag
WB: Anti-Flag

Fig. 4

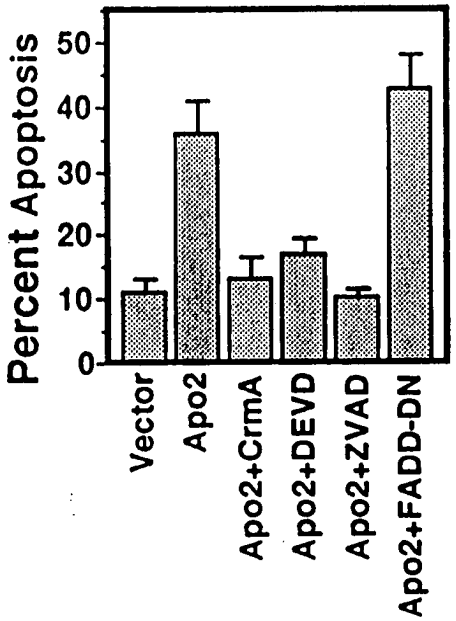
4A



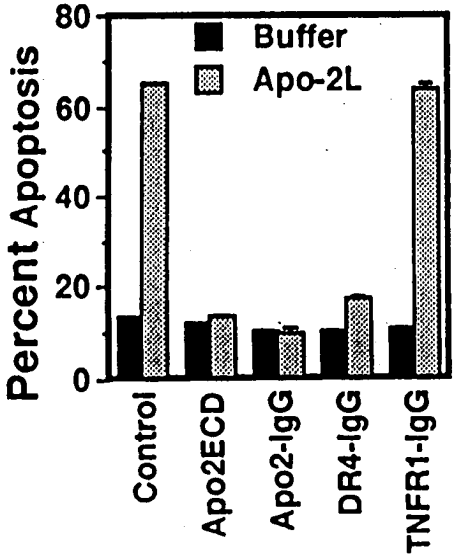
4B



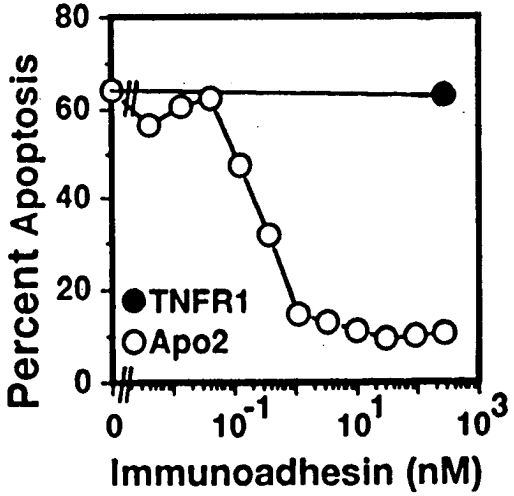
4C



4D



4E



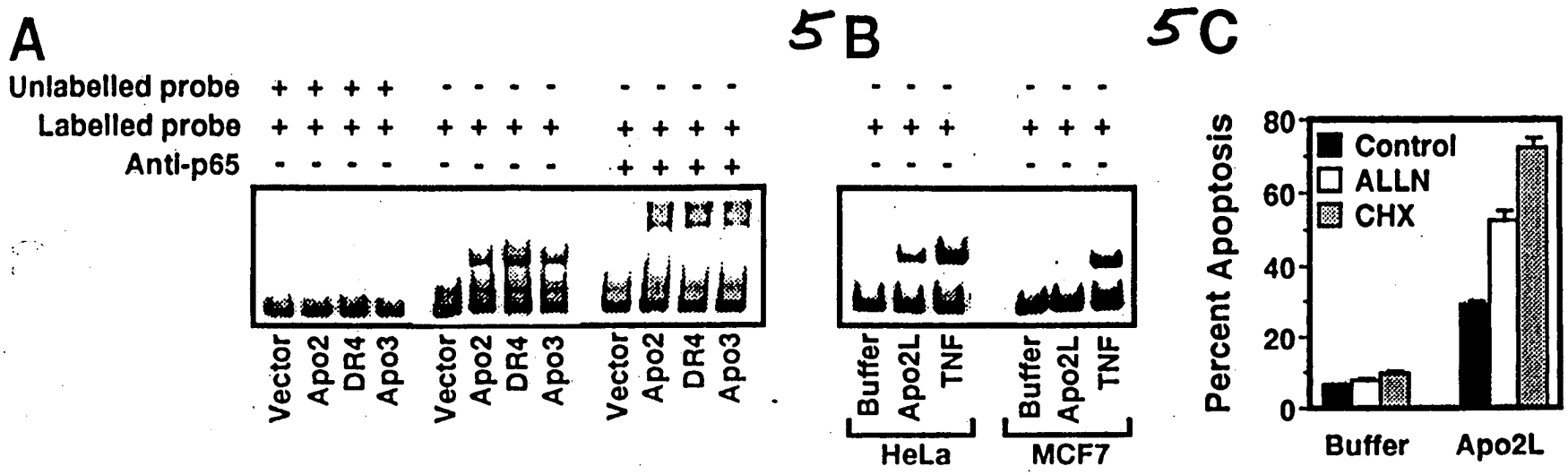

5A
5B
5C
Unlabelled probe
Labelled probe
Anti-p65
Vector
Apo2
DR4
Apo3
Buffer
Apo2L
TNF
HeLa
MCF7
Percent Apoptosis
Control
ALLN
CHX
Buffer
Apo2L

FIG. 5

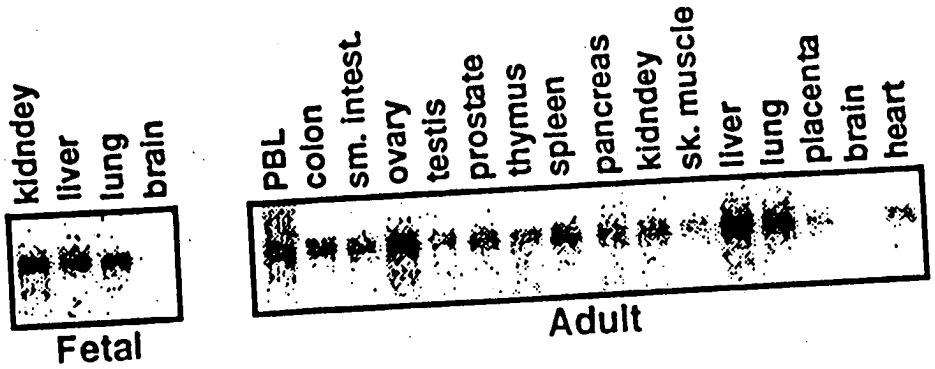

kidndey
liver
lung
brain
Fetal
PBL
colon
sm. intest.
ovary
testis
prostate
thymus
spleen
pancreas
kidndey
sk. muscle
liver
lung
placenta
brain
heart
Adult

FIG. 6

GH-50008-1

Tumor Necrosis Factor Related Receptor, TR6

This application is a continuation-in-part application of U.S. Serial No: 08/853,684, filed May 9, 1997, now abandoned which claims the benefit of U.S. Provisional Application No: 60/041,230, filed March 14, 1997.

FIELD OF INVENTION

This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to Tumor Necrosis Factor Related family, hereinafter referred to as TR6. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

BACKGROUND OF THE INVENTION

Many biological actions, for instance, response to certain stimuli and natural biological processes, are controlled by factors, such as cytokines. Many cytokines act through receptors by engaging the receptor and producing an intracellular response.

For example, tumor necrosis factors (TNF) alpha and beta are cytokines which act through TNF receptors to regulate numerous biological processes, including protection against infection and induction of shock and inflammatory disease. The TNF molecules belong to the "TNF-ligand" superfamily, and act together with their receptors or counter-ligands, the "TNF-receptor" superfamily. So far, nine members of the TNF ligand superfamily have been identified and ten members of the TNF-receptor superfamily have been characterized.

Among the ligands there are included TNF-α, lymphotoxin-α (LT-α, also known as TNF-β), LT-β (found in complex heterotrimer LT-α2-β), FasL, CD40L, CD27L, CD30L, 4-1BBL, OX40L and nerve growth factor (NGF)). The superfamily of TNF receptors includes the p55TNF receptor, p75TNF receptor, TNF receptor-related protein, FAS antigen or APO-1, CD40, CD27, CD30, 4-1BB, OX40, low affinity p75 and NGF-receptor (Meager, A., Biologicals, 22:291-295 (1994)).

Many members of the TNF-ligand superfamily are expressed by activated T-cells,

implying that they are necessary for T-cell interactions with other cell types which underlie cell ontogeny and functions. (Meager, A., supra).

Considerable insight into the essential functions of several members of the TNF receptor family has been gained from the identification and creation of mutants that abolish the expression of these proteins. For example, naturally occurring mutations in the FAS antigen and its ligand cause lymphoproliferative disease (Watanabe-Fukunaga, R., et al., Nature 356:314 (1992)), perhaps reflecting a failure of programmed cell death. Mutations of the CD40 ligand cause an X-linked immunodeficiency state characterized by high levels of immunoglubulin M and low levels of immunoglobulin G in plasma, indicating faulty T-cell-dependent B-cell activation (Allen, R.C. et al., Science 259:990 (1993)). Targeted mutations of the low affinity nerve growth factor receptor cause a disorder characterized by faulty sensory innovation of peripheral structures (Lee, K.F. et al, Cell 69:737 (1992)).

TNF and LT-α are capable of binding to two TNF receptors (the 55- and 75-kd TNF receptors). A large number of biological effects elicited by TNF and LT-α, acting through their receptors, include hemorrhagic necrosis of transplanted tumors, cytotoxicity, a role in endotoxic shock, inflammation, immunoregulation, proliferation and anti-viral responses, as well as protection against the deleterious effects of ionizing radiation. TNF and LT-α are involved in the pathogenesis of a wide range of diseases, including endotoxic shock, cerebral malaria, tumors, autoimmuine disease, AIDS and graft-host rejection (Beutler, B. and Von Huffel, C., Science 264:667-668 (1994)). Mutations in the p55 Receptor cause increased susceptibility to microbial infection.

Moreover, an about 80 amino acid domain near the C-terminus of TNFR1 (P55) and Fas was reported as the "death domain," which is responsible for transducing signals for programmed cell death (Tartaglia et al., Cell 74:845 (1993)).

The effects of TNF family ligands and TNF family receptors are varied and influence numerous functions, both normal and abnormal, in the biological processes of the mammalian system. There is a clear need, therefore, for identification and characterization of such receptors and ligands that influence biological activity, both normally and in disease states. In particular, there is a need to isolate and characterize novel members of the TNF receptor family.

This indicates that these receptors have an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further receptors which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to TR6 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such TR6 polypeptides and polynucleotides. Such uses include the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with TR6 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate TR6 activity or levels.

DESCRIPTION OF THE INVENTION

Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"TR6" refers, among others, to a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2, or an allelic variant thereof.

"Receptor Activity" or "Biological Activity of the Receptor" refers to the metabolic or physiologic function of said TR6 including similar activities or improved

activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said TR6.

"TR6 gene" refers to a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO:1 or allelic variants thereof and/or their complements.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein

cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two

polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., *et al.*, *Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. *et al.*, *J Molec Biol* (1990) 215:403).

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO: 1 is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO: 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5 or 3 terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference amino acid sequence of SEQ ID NO:2 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO: 2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may

be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

Polypeptides of the Invention

In one aspect, the present invention relates to TR6 polypeptides. The TR6 polypeptides include the polypeptides of SEQ ID NOS:2 and 4; as well as polypeptides comprising the amino acid sequence of SEQ ID NO:2; and polypeptides comprising the amino acid sequence which have at least 80% identity to that of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Also included within TR6 polypeptides are polypeptides having the amino acid sequence which have at least 80% identity to the polypeptide having the amino acid sequence of SEQ ID NO: 2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Preferably TR6 polypeptides exhibit at least one biological activity of the receptor.

The TR6 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

Fragments of the TR6 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned TR6 polypeptides. As with TR6 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of

polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of TR6 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of TR6 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate receptor activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Preferably, all of these polypeptide fragments retain the biological activity of the receptor, including antigenic activity. Among the most preferred fragment is that having the amino acid sequence of SEQ ID NO: 4. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions – i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

The TR6 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

Another aspect of the invention relates to TR6 polynucleotides. TR6 polynucleotides include isolated polynucleotides which encode the TR6 polypeptides and fragments, and polynucleotides closely related thereto. More specifically, TR6 polynucleotide of the invention include a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO:1 encoding a TR6 polypeptide of SEQ ID NO: 2, and polynucleotides having the particular sequences of SEQ ID NOS:1 and 3. TR6 polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the TR6 polypeptide of SEQ ID NO:2 over its entire length, and a polynucleotide that is at least 80% identical to that having SEQ ID NO:1 over its entire length. In this regard, polynucleotides at least 90% identical are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under TR6 polynucleotides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such TR6 polynucleotides.

TR6 of the invention is structurally related to other proteins of the Tumor Necrosis Factor Related family, as shown by the results of sequencing the cDNA encoding human TR6. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide numbers 94 to 1329) encoding a polypeptide of 411 amino acids of SEQ ID NO:2. The amino acid sequence of Table 1 (SEQ ID NO:2) has about 58% identity (using GAP (From GCG)) in 411 amino acid residues with DR4, the receptor for the ligand TRAIL. (Pan,G., O'Rourke,K., Chinnaiyan,A.M., Gentz,R., Ebner,R., Ni,J. and Dixit,V.M., Science 276, 111-113 (1997)). The nucleotide sequence of Table 1 (SEQ ID NO:1) has about 70% identity (using GAP (from GCG)) in 1335 nucleotide residues with DR4, the receptor for the ligand TRAIL. TR6 contains a death domain (amino acids 290 to 324 in SEQ ID NO:2) which is 64% identical to the death domain of the human Death receptor 4 (DR4) (Pan,G., O'Rourke,K.,

Chinnaiyan,A.M., Gentz,R., Ebner,R., Ni,J. and Dixit,V.M., Science 276, 111-113 (1997)), 35.7% identical to the death domain of the human Death receptor 3 (DR3) (A.M. Chinnaiyan, et al, Science 274 (5289), 990-992 (1996)), 32.7% identical to the death domain of human TNFR-1, and 19.6% identical to the death domain of CD95 (Fas) (I. Cascino, J. Immunol. 154 (6), 2706-2713 (1995)).

Table 1*

```
   1  CTTTGCGCCC ACAAAATACA CCGACGATGC CCGATCTACT TTAAGGGCTG
  51  AAACCCACGG GCCTGAGAGA CTATAAGAGC GTTCCCTACC GCCATGGAAC
 101  AACGGGGACA GAACGCCCCG GCCGCTTCGG GGGCCCGGAA AAGGCACGGC
 151  CCAGGACCCA GGGAGGCGCG GGGAGCCAGG CCTGGGCCCC GGGTCCCCAA
 201  GACCCTTGTG CTCGTTGTCG CCGCGGTCCT GCTGTTGGTC TCAGCTGAGT
 251  CTGCTCTGAT CACCCAACAA GACCTAGCTC CCCAGCAGAG AGCGGCCCCA
 301  CAACAAAAGA GGTCCAGCCC CTCAGAGGGA TTGTGTCCAc cTGGACACCA
 351  TATCTCAGAA GACGGTAGAG ATTGCATCTC CTGCAAATAT gGACAGGACT
 401  ATAGCACTCA aTGGAATGAC CTCCTTTTCT GCTTGCGCTG CACCAGGTGT
 451  GATTCAGGTG AAGTGGAGCT AAGTCCCTGC ACCACGACCA GAAACACAGT
 501  GTGTCAGTGC GAAGAAgGCA CCTTCCGGGA AGAAGATTCT CCTGAGATGT
 551  GCCGGAAGTG CCGCACAGGG TGTCCCAgAG GGATGGTCAA GGTCGGTGAT
 601  TGTACACCCT GGAGTGACAT CGAATGTGTC CACAAAGAAT CAGGCATCAT
 651  CATAgGAGTC ACAGTTGCAG CCGTAGTCTT GATTGTGGCT GTGTTTGTTT
 701  GCaAgTCTTT ACTGTGGAAg AAAGTCCTTC CTTACCTGAA AGGCATCTGC
 751  TCAGGTGGTG GTGGGGACCC TGAGCGTGTG GACAGAAGcT CACAACGACc
```

```
 801  TGGGGCTGAG GACAATGTCC TCAATGAGAT CGTGAGTATC TTGCAGCCCA

 851  CCCAGGTCCC TGAGCAGGAA ATGGAAGTCC AGGAGCCAGC AGAGCCAACA

 901  GGTGTCAACA TGTTGTCCCC CGGGGAGTCA GAGCATCTGC TGGAACCGGC

 951  AGAAGCTGAA AGGTCTCAGA GGAGGAGGCT GCTGGTTCCA GCAAATGAAG

1001  GTGATCCCAC TGAGACTCTG AGACAGTGCT TCGATGACTT TGCAGACTTG

1051  GTGCCCTTTG ACTCCTGGGA gCCgCTCATG AGGAAGTTGG GCCTCATGGA

1101  CAATgAGATa aaGGTGGCTA AAGCTGAGGC AGCGGGCCAC AGGGACACCT

1151  TGTACACGAT GCTGATAAAG TGGGTCAACA AAACCGGGCG AGATGCCTCT

1201  GTCCACACCC TGCTGGATGC CTTGGAGACG CTGGGAGAGA GACTTGCCAA

1251  GCAGAAGATT GAGGACCACT TGTTGAGCTC TGGAAAGTTC ATGTATCTAG

1301  AAGGTAATGC AGACTCTGCC ATGTCCTAAG TGTGATTCTC TTCAGGAAGT

1351  CAGACCTTCC CTGGTTTACC TTTTTTCTGG AAAAAGCCCA ACTGGACTCC

1401  AGTCAGTAGG AAAGTGCCAC AATTGTCACA TGACCGGTAC TGGAAGAAAC

1451  TCTCCCATCC AACATCACCC AGTGGATGGA ACATCCTGTA ACTTTTCACT

1501  GCACTTGGCA TTATTTTTAT AAGCTGAATG TGATAATAAG GACACTATGG

1551  AAATGTCTGG ATCATTCCGT TTGTGCGTAC TTTGAgATTT GGTTTGGGAT

1601  GTCATTGTTT TCACAGCACT TTTTTATCCT AATGTAAATG CTTTATTTAT

1651  TTATTTGGGC TACATTGTAA gATCCATCTA CACAGTCGTT GTCCGACTTC

1701  ACTTGATACT ATATGATATG AACCTTTTTT GGGTGGGGGG TGCGGGGCAg

1751  TTCACTCTGT CTCCCAGGCT GGAGTGCAAT GGTGCAATCT TGGCTCACTA

1801  TAGCCTTGAC CTCTCAGGCT CAAGCGATTC TCCCACCTCA GCCATCCAAA
```

```
1851  TAGCTGGGAC CACAGGTGTG CACCACCACG CCCGGCTAAT TTTTTGTATT
1901  TTGTCTAgAT ATAGGGGCTC TCTATGTTGC TCAGGGTGGT CTCgAATTCC
1951  TGGAcTCAAG CAGTCTGCCC ACcTCAGAcT CCCAAAGCGG TGGAATTAGA
2001  GGCGTGAGCC CCCATGcTTG gCCTTACcTT TcTACTTTTA TAATTCTGTA
2051  TGTTATTATT TTATGAACAT GAAGAAACTT TAGTAAATGT ACTTGTTTAC
2101  ATAGTTATGT GAATAGATTA GATAAACATA AAAGGAGGAG ACATACAATG
2151  GGGGAAGAAG AAGAAGTCCC CTGTAAGATG TCACTGTcTG GGTTCCAGCC
2201  CTCCCTCAGA TGTACTTTGG CTTCAATGAT TGGCAACTTC TACAGGGGCC
2251  AGTCTTTTGA ACTGGACAAC CTTACAAGTA TATGAGTATT ATTTATAGGT
2301  AGTTGTTTAC ATATGAGTCG GGACCAAAGA GAACTGGATC CACGTGAAGT
2351  CCTGTGTGTG GCTGGTCCCT ACCTGGGCAG TcTCATTTGC ACCCATAGCC
2401  CCCATCTATG GACAGGCTGG GACAGAGGCA GATGGGTTAG ATCACACATA
2451  ACAATAGGGT CTATGTCATA TCCCAAGTGA ACTTGAGCCC TGTTTGGGCT
2501  CAGGAGATAG AAGACAAAAT CTGTCTCCCC ACGTCTGCCA TGGCATCAAG
2551  GGGGAAGAGT AGATGGTGCT tGAGAATGGT GTGAAATGGT TGCCATCTCA
2601  GGAGTAGATG GCCCGGCTCA CTTCTGGTTA TCtGTCACCC TGAGCCCAtG
2651  AGCTGCcTTT TAGGGTACAG ATTGCCTACT TGAGGACCTT GGCCGCTCTG
2701  TAAGCATCTG ACTCATCTCA GAAATGTCAA TTCTTAAACA CTGTGGCAAC
2751  AGGACCTAGA ATGGCTGACG CATTAAGGTT TTCTTcTTGT GTCCTGTTCT
2801  ATTAttGTTT TAAGACCTCA GTAACCATTT CAGCCTCTTT CCAGCAAACC
```

```
2851  CTTCTCCATA GTATTTCAGT CATGGAAGGA TCATTTATGC AGGTAGTCAT

2901  TCCAGGAGTT TTTGGTCTTT TCTGTCTCAA GGCATTGTGT GTTTTGTTCC

2951  GGCACTGGTT TGGGTGGGAC AAAGTTAGAA TTGCCTGAAG ATCACACATT

3001  CAGACTGTCG TGTCTGTGGA GTTTTAGGAG TGGGGGGTGA CCTTTCTGGT

3051  CTTTGCACTT CCATCCTCTC CCACTTCCAT CTGGCATCCC CACGCGTTGT

3101  CCCCTGCACT TCTGGAAGGC ACAGGGTGCT GCTGCTTCCT GGTCTTTGCC

3151  TTTGCTGGGC CTTCTGTGCA GGACGCTCAG CCTCAGGGCT CAGAAGGTGC

3201  CAGTCCGGTC CCAGGTCCCT TGTCCCTTCC ACAGAGGCCT TCCTAGAAGA

3251  TGCATCTAGA GTGTCAGCCT TATCAGTGTT TAAGATTTTT CTTTTATTTT

3301  TAATTTTTTT GAGACAGAAT CTCACTCTCT CGCCCAGGCT GGAGTGCAAC

3351  GGTACGATCT TGGCTCAGTG CAACCTCCGC CTCCTGGGTT CAAGCGATTC

3401  TCGTGCCTCA GCCTCCGGAG TAGCTGGGAT TGCAGGCACC CGCCACCACG

3451  CCTGGCTAAT TTTTGTATTT TTAGTAGAGA CGGGGTTTCA CCATGTTGGT

3501  CAGGCTGGTC TCGAACTCCT GACCTCAGGT GATCCACNTT GGCCTCCGAA

3551  AGTGCTGGGa tatacaaggc GTGAGCCACC AGCCAGGCCA AGATATTNTT

3601  NTAAAGNNAG CTTCCGGANG ACATGAAATA ANGGGGGGTT TTGTTGTTTA

3651  GTAACATTNG GCTTTGATAT ATCCCCAGGC CAAATNGCAN GNGACACAGG

3701  ACAGCCATAG TATAGTGTGT CACTCGTGGT TGGTGTCCTT TCATGGTTCT

3751  GCCCTGTCAA AGGTCCCTAT TTGAAATGTG TTATAATACA AACAAGGAAG

3801  CACATTGTGT ACAAAATACT TATGTATTTA TGAATCCATG ACCAAATTAA

3851  ATATGAAACC TTATATAAAA AAAAAAAAAA A
```

[redacted]

[a] A nucleotide sequence of a human TR6. (SEQ ID NO: 1).

Table 2[b]

1	Met Glu Gln Arg Gly Gln Asn Ala Pro Ala Ala Ser Gly Ala Arg Lys	16
17	Arg His Gly Pro Gly Pro Arg Glu Ala Arg Gly Ala Arg Pro Gly Pro	32
33	Arg Val Pro Lys Thr Leu Val Leu Val Val Ala Ala Val Leu Leu Leu	48
49	Val Ser Ala Glu Ser Ala Leu Ile Thr Gln Gln Asp Leu Ala Pro Gln	64
65	Gln Arg Ala Ala Pro Gln Gln Lys Arg Ser Ser Pro Ser Glu Gly Leu	80
81	Cys Pro Pro Gly His His Ile Ser Glu Asp Gly Arg Asp Cys Ile Ser	96
97	Cys Lys Tyr Gly Gln Asp Tyr Ser Thr Gln Trp Asn Asp Leu Leu Phe	112
113	Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val Glu Leu Ser Pro	128
129	Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu Glu Gly Thr Phe	144
145	Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys Arg Thr Gly Cys	160
161	Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile	176
177	Glu Cys Val His Lys Glu Ser Gly Ile Ile Ile Gly Val Thr Val Ala	192
193	Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp	208
209	Lys Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly	224
225	Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp	240
241	Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val Pro	256
257	Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly Val Asn	272
273	Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala Glu Ala	288

289	Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala Asn Glu Gly Asp	304
305	Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe Ala Asp Leu Val	320
321	Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu Gly Leu Met Asp	336
337	Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly His Arg Asp Thr	352
353	Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr Gly Arg Asp Ala	368
369	Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu Gly Glu Arg Leu	384
385	Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser Gly Lys Phe Met	400
401	Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser End	411

An amino acid sequence of a human TR6. (SEQ ID NO: 2).

One polynucleotide of the present invention encoding TR6 may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of human of human thymus stromal cells, monocytes, peripheral blood lymphocytes, primary dendritic, and bone marrow cells using the expressed sequence tag (EST) analysis (Adams, M.D., *et al. Science* (1991) 252:1651-1656; Adams, M.D. *et al., Nature*, (1992) *355*:632-634; Adams, M.D., *et al., Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

The nucleotide sequence encoding TR6 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in Table 1 (nucleotide number 94 to 1329 of SEQ ID NO:1), or it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of TR6 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or

secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further preferred embodiments are polynucleotides encoding TR6 variants comprising the amino acid sequence of TR6 polypeptide of Table 1 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination. Among the preferred polynucleotides of the present invention is contained in Table 3 (SEQ ID NO: 3) encoding the amino acid sequence of Table 4 (SEQ ID NO: 4).

Table 3*

```
   1  ATGACCTCCT TTTCTGCTTG CGCTGCACCA GGTGTGATTC AGGTGAAGTG

  51  GAGCTAAGTC CCTGCACCAC GACCAGAAAC ACAGTGTGTC AGTGCGAAGA

 101  AgGCACCTTC CGGGAAGAAG ATTCTCCTGA GATGTGCCGG AAGTGCCGCA

 151  CAGGGTGTCC CAgAGGGATG GTCAAGGTCG GTGATTGTAC ACCCTGGAGT

 201  GACATCGAAT GTGTCCACAA AGAATCAGGC ATCATCATAg GAGTCACAGT

 251  TGCAGCCGTA GTCTTGATTG TGGCTGTGTT TGTTTGCaAg TCTTTACTGT

 301  GGAAgAAAGT CCTTCCTTAC CTGAAAGGCA TCTGCTCAGG TGGTGGTGGG

 351  GACCCTGAGC GTGTGGACAG AAGcTCACAA CGACcTGGGG CTGAGGACAA

 401  TGTCCTCAAT GAGATCGTGA GTATCTTGCA GCCCACCCAG GTCCCTGAGC

 451  AGGAAATGGA AGTCCAGGAG CCAGCAGAGC CAACAGGTGT CAACATGTTG

 501  TCCCCCGGGG AGTCAGAGCA TCTGCTGGAA CCGGCAGAAG CTGAAAGGTC
```

```
 551  TCAGAGGAGG AGGCTGCTGG TTCCAGCAAA TGAAGGTGAT CCCACTGAGA

 601  CTCTGAGACA GTGCTTCGAT GACTTTGCAG ACTTGGTGCC CTTTGACTCC

 651  TGGGAgCCgC. TCATGAGGAA GTTGGGCCTC ATGGACAATg AGATnnnGGT

 701  GGCTAAAGCT GAGGCAGCGG GCCACAGGGA CACCTTGTAC ACGATGCTGA

 751  TAAAGTGGGT CAACAAAACC GGGCGAGATG CCTCTGTCCA CACCCTGCTG

 801  GATGCCTTGG AGACGCTGGG AGAGAGACTT GCCAAGCAGA AGATTGAGGA

 851  CCACTTGTTG AGCTCTGGAA AGTTCATGTA TCTAGAAGGT AATGCAGACT

 901  CTGCCATGTC CTAAGTGTGA TTCTCTTCAG GAAGTCAGAC CTTCCCTGGT

 951  TTACCTTTTT TCTGGAAAAA GCCCAACTGG ACTCCAGTCA GTAGGAAAGT

1001  GCCACAATTG TCACATGACC GGTACTGGAA GAAACTCTCC CATCCAACAT

1051  CACCCAGTGG AT
```

[a] A partial nucleotide sequence of a human TR6. (SEQ ID NO: 3).

Table 4[d]

```
   1  DLLPCLRCTR CDSGEVELSP CTTTRNTVCQ CEEGTFREED SPEMCRKCRT

  51  GCPRGMVKVG DCTPWSDIEC VHKESGIIIG VTVAAVVLIV AVFVCKSLLW

 101  KKVLPYLKGI CSGGGGDPER VDRSSQRPGA EDNVLNEIVS ILQPTQVPEQ

 151  EMEVQEPAEP TGVNMLSPGE SEHLLEPAEA ERSQRRRLLV PANEGDPTET

 201  LRQCFDDFAD LVPFDSWEPL MRKLGLMDNE IKVAKAEAAG HRDTLYTMLI

 251  KWVNKTGRDA SVHTLLDALE TLGERLAKQK IEDHLLSSGK FMYLEGNADS

 301  AMS*
```

[d] A partial amino acid sequence of a human TR6. (SEQ ID NO: 4).

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences.

Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1 or a fragment thereof, including that of SEQ ID NO:3, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding TR6 and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the TR6 gene. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

In one embodiment, to obtain a polynucleotide encoding TR6 polypeptide comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof, including that of SEQ ID NO: 3, and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to those of skill in the art. Thus in another aspect, TR6 polynucleotides of the present invention further include a nucleotide sequence comprising a nucleotide sequence that hybridize under stringent condition to a nucleotide sequence having SEQ ID NO: 1 or a fragment thereof, including that of SEQ ID NO:3. Also included with TR6 polypeptides are polypeptide comprising amino acid sequence encoded by nucleotide sequence obtained by the above hybridization condition. Stringent hybridization conditions are as defined above or alternatively conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

Vectors, Host Cells, Expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and

bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL* (*supra*).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If the TR6 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If TR6 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

TR6 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention also relates to the use of TR6 polynucleotides for use as diagnostic reagents. Detection of a mutated form of TR6 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of TR6. Individuals carrying mutations in the TR6 gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled TR6 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al.*, *Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al.*, *Proc Natl Acad Sci USA* (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotides probes comprising TR6 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee et al., Science, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease, through detection of mutation in the TR6 gene by the methods described.

In addition, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease, can be diagnosed by methods comprising determining from a sample derived from a

subject an abnormally decreased or increased level of TR6 polypeptide or TR6 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an TR6, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes). The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

The 3' untranslated region of TR6 matches the 295 bp nucleotide sequence of a mapped EST (Genbank ID: D20151). This EST has been mapped by the Whitehead Institute to chromosome 8, 97.68 cR from the top of the Chromosome 8 linkage group

Chromosome 8.
TR6 3' UTR.

Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the TR6 polypeptides. The term "immunospecific" means that the antibodies have substantiall greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the TR6 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, ·genic mice, or other organisms including other mammals, may be used to express ·ed antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against TR6 polypeptides may also be employed to treat chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease, among others.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with TR6 polypeptide, or

a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease, among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering TR6 polypeptide via a vector directing expression of TR6 polynucleotide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a TR6 polypeptide wherein the composition comprises a TR6 polypeptide or TR6 gene. The vaccine formulation may further comprise a suitable carrier. Since TR6 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation instonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

We have now discovered that TL2 of SEQ ID NO: 5 (otherwise known as TRAIL, Immunity (6):673-682 (1995)) is a ligand of TR6. Thus, the TR6 polypeptide of the present

↑ Is is this.

invention, and one of its ligands, TL2 may be employed in a screening process for compounds which bind the receptor, or its ligand, and which activate (agonists) or inhibit activation of (antagonists) the receptor polypeptide of the present invention, or its ligand TL2. Thus, polypeptides of the invention may be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

TR6 polypeptides are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate TR6 on the one hand and which can inhibit the function of TR6 or remove TR6 expressing cells on the other hand. Antagonists, or agents which remove TR6 expressing cells, may be employed for a variety of therapeutic and prophylactic purposes for such conditions as chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease. Agonists can be employed for therapeutic and prophylactic purposes for such conditions responsive to activation of T cells and other components of the immune system, such as for treatment of cancer and AIDS. However, agonists can also be employed for inappropriate stimulation of T cells and other components of the immune system which leads to down modulation of immune activity with therapeutic or prophylactic application for conditions such , as chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, , Bone diseases,, atheroschlerosis, and Alzheimers disease.

Candidate compounds may be identified using assays to detect compounds which inhibit binding of TL2 to TR6 in either cell-free or cell based assays. Suitable cell-free assays may be readily determined by one of skill in the art. For example, an ELISA format may be used in which purified TR6, or a purified derivative of TR6, containing the extracellular

domain of TR6, is immobilized on a suitable surface, either directly or indirectly (e.g., via an antibody to TR6) and candidate compounds are identified by their ability to block binding of purified TL2 to TR6. The binding of TL2 to TR6 could be detected by using a label directly or indirectly associated with TL2. Suitable detection systems include the streptavidin horseradish peroxidase conjugate, or direct conjugation by a tag, e.g., fluorescein. Conversely, purified TL2 may be immobilized on a suitable surface, and candidate compounds identified by their ability to block binding of purified TR6 to TL2. The binding of TR6 to TL2 could be detected by using a label directly or indirectly associated with TR6. Many other assay formats are possible that use the TR6 protein and its ligands.

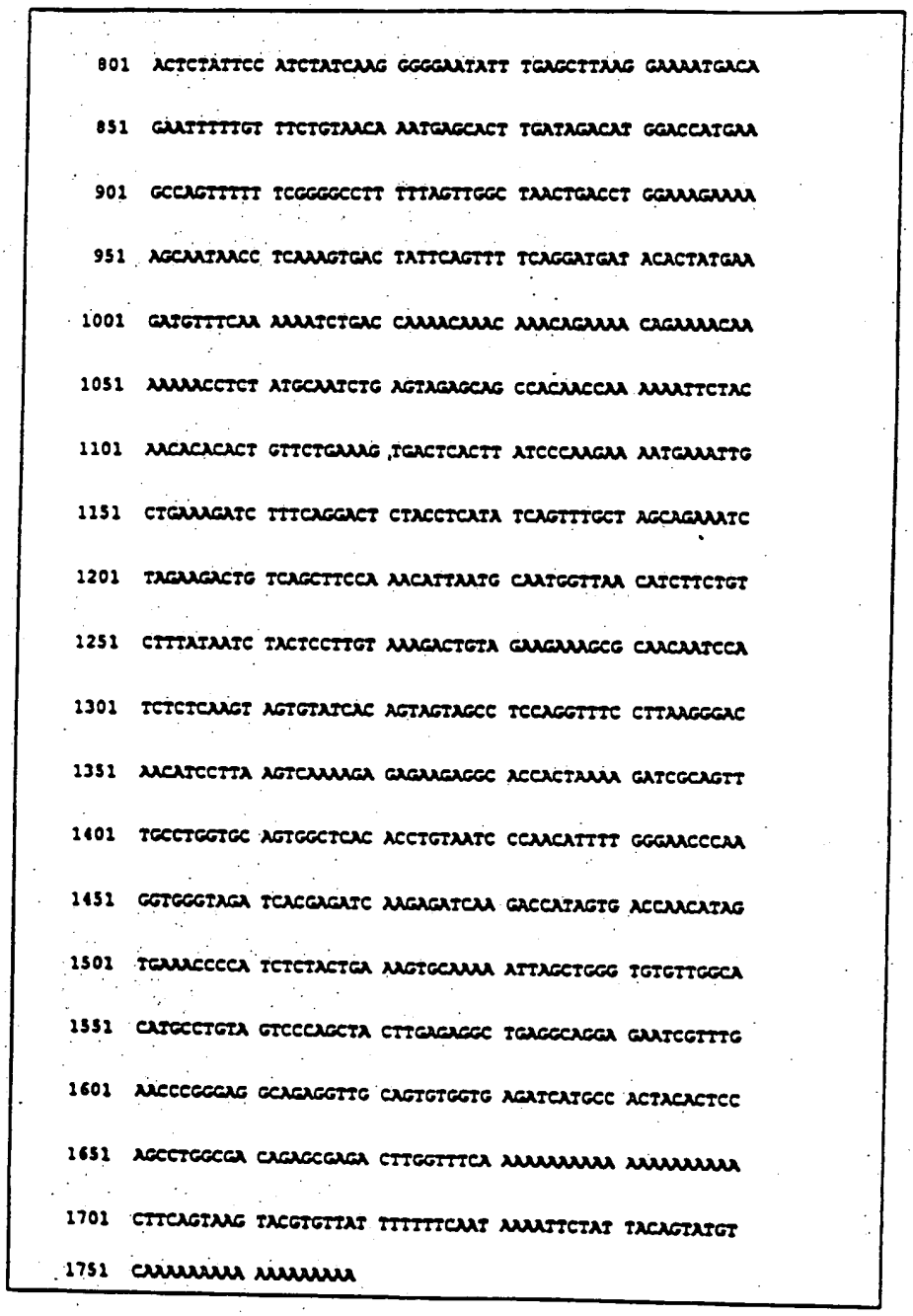
Suitable cell based assays may be readily determined by one of skill in the art. In general, such screening procedures involve producing appropriate cells which express the receptor polypeptide of the present invention on the surface thereof. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells expressing the receptor (or cell membrane containing the expressed receptor) are then contacted with a known ligand, such as TL2, or test compound to observe binding, or stimulation or inhibition of a functional response. The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the receptor is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor, such as the ligand TL2. Further, these assays may test whether the candidate compound results in a signal generated by activation of the receptor or its ligand (e.g. TL2)using detection systems appropriate to the cells bearing the receptor or its ligand and fusion proteins thereof at their surfaces. Typical fusion partners include fusing the extracellular domain of the receptor or ligand with the intracellular tyrosine kinase domain of a second receptor. Inhibitors of activation are generally assayed in the presence of a known agonist, such as the ligand TL2, and the effect on activation by the agonist by the presence of the candidate compound is observed. Standard methods for conducting such screening assays are well understood in the art.

Examples of potential TR6 antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligand of the TR6, e.g., a fragment of the ligand TL2, or small molecules which bind to the receptor, or its ligand, but do not elicit

a response, so that the activity of the receptor is prevented. Examples of potential TR6 agonists include antibodies that bind to TR6, its ligand, such as TL2, or derivatives thereof, and small molecules that bind to TR6. These agonists will elicit a response mimicking all or part of the response induced by contacting the native ligand.

The nucleotide sequence of TL2 (SEQ ID NO:5) (published by Immunex Research and Development Corporation, Seattle, Washington as TNF-related apoptosis-inducing ligand (TRAIL) TWiley SR, et al. Immunity (6):673-682 (1995)) is as follows.

```
   1  CCTCACTGAC TATAAAAGAA TAGAGAAGGA AGGGCTTCAG TGACCGGCTG

  51  CCTGGCTGAC TTACAGCAGT CAGACTCTGA CAGGATCATG GCTATGATGG

 101  AGGTCCAGGG GGGACCCAGC CTGGGACAGA CCTGCGTGCT GATCGTGATC

 151  TTCACAGTGC TCCTGCAGTC TCTCTGTGTG GCTGTAACTT ACGTGTACTT

 201  TACCAACGAG CTGAAGCAGA TGCAGGACAA GTACTCCAAA AGTGGCATTG

 251  CTTGTTTCTT AAAAGAAGAT GACAGTTATT GGGACCCCAA TGACGAAGAG

 301  AGTATGAACA GCCCCTGCTG GCAAGTCAAG TGGCAACTCC GTCAGCTCGT

 351  TAGAAAGATG ATTTTGAGAA CCTCTGAGGA AACCATTTCT ACAGTTCAAG

 401  AAAAGCAACA AAATATTTCT CCCCTAGTGA GAGAAAGAGG TCCTCAGAGA

 451  GTAGCAGCTC ACATAACTGG GACCAGAGGA AGAAGCAACA CATTGTCTTC

 501  TCCAAACTCC AAGAATGAAA AGGCTCTGGG CCGCAAAATA AACTCCTGGG

 551  AATCATCAAG GAGTGGGCAT TCATTCCTGA GCAACTTGCA CTTGAGGAAT

 601  GGTGAACTGG TCATCCATGA AAAAGGGTTT TACTACATCT ATTCCCAAAC

 651  ATACTTTCGA TTTCAGGAGG AAATAAAAGA AAACACAAAG AACGACAAAC

 701  AAATGGTCCA ATATATTTAC AAATACACAA GTTATCCTGA CCCTATATTG

 751  TTGATGAAAA GTGCTAGAAA TAGTTGTTGG TCTAAAGATG CAGAATATGG
```



```
 801  ACTCTATTCC ATCTATCAAG GGGGAATATT TGAGCTTAAG GAAAATGACA
 851  GAATTTTTGT TTCTGTAACA AATGAGCACT TGATAGACAT GGACCATGAA
 901  GCCAGTTTTT TCGGGGCCTT TTTAGTTGGC TAACTGACCT GGAAAGAAAA
 951  AGCAATAACC TCAAAGTGAC TATTCAGTTT TCAGGATGAT ACACTATGAA
1001  GATGTTTCAA AAAATCTGAC CAAAACAAAC AAACAGAAAA CAGAAAACAA
1051  AAAAACCTCT ATGCAATCTG AGTAGAGCAG CCACAACCAA AAAATTCTAC
1101  AACACACACT GTTCTGAAAG TGACTCACTT ATCCCAAGAA AATGAAATTG
1151  CTGAAAGATC TTTCAGGACT CTACCTCATA TCAGTTTGCT AGCAGAAATC
1201  TAGAAGACTG TCAGCTTCCA AACATTAATG CAATGGTTAA CATCTTCTGT
1251  CTTTATAATC TACTCCTTGT AAAGACTGTA GAAGAAAGCG CAACAATCCA
1301  TCTCTCAAGT AGTGTATCAC AGTAGTAGCC TCCAGGTTTC CTTAAGGGAC
1351  AACATCCTTA AGTCAAAAGA GAGAAGAGGC ACCACTAAAA GATCGCAGTT
1401  TGCCTGGTGC AGTGGCTCAC ACCTGTAATC CCAACATTTT GGGAAGCCAA
1451  GGTGGGTAGA TCACGAGATC AAGAGATCAA GACCATAGTG ACCAACATAG
1501  TGAAACCCCA TCTCTACTGA AAGTGCAAAA ATTAGCTGGG TGTGTTGGCA
1551  CATGCCTGTA GTCCCAGCTA CTTGAGAGGC TGAGGCAGGA GAATCGTTTG
1601  AACCCGGGAG GCAGAGGTTG CAGTGTGGTG AGATCATGCC ACTACACTCC
1651  AGCCTGGCGA CAGAGCGAGA CTTGGTTTCA AAAAAAAAAA AAAAAAAAAA
1701  CTTCAGTAAG TACGTGTTAT TTTTTTCAAT AAAATTCTAT TACAGTATGT
1751  CAAAAAAAAA AAAAAAAAA
```

The amino acid sequence of TL2 (SEQ ID NO:6) (published by Immunex Research and Development Corporation, Seattle, Washington as TNF-related apoptosis-inducing ligand (TRAIL) TWiley SR, et al. Immunity (6):673-682 (1995)) is as follows:

1	Met Ala Met Met Glu Val Gln Gly Gly Pro Ser Leu Gly Gln Thr Cys	16
17	Val Leu Ile Val Ile Phe Thr Val Leu Leu Gln Ser Leu Cys Val Ala	32
33	Val Thr Tyr Val Tyr Phe Thr Asn Glu Leu Lys Gln Met Gln Asp Lys	48
49	Tyr Ser Lys Ser Gly Ile Ala Cys Phe Leu Lys Glu Asp Asp Ser Tyr	64
65	Trp Asp Pro Asn Asp Glu Glu Ser Met Asn Ser Pro Cys Trp Gln Val	80
81	Lys Trp Gln Leu Arg Gln Leu Val Arg Lys Met Ile Leu Arg Thr Ser	96
97	Glu Glu Thr Ile Ser Thr Val Gln Glu Lys Gln Gln Asn Ile Ser Pro	112
113	Leu Val Arg Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile Thr Gly	128
129	Thr Arg Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu	144
145	Lys Ala Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly	160
161	His Ser Phe Leu Ser Asn Leu His Leu Arg Asn Gly Glu Leu Val Ile	176
177	His Glu Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe	192
193	Gln Glu Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val Gln	208
209	Tyr Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys	224
225	Ser Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr	240
241	Ser Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile	256
257	Phe Val Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His Glu Ala	272
273	Ser Phe Phe Gly Ala Phe Leu Val Gly End	281

Prophylactic and Therapeutic Methods

This invention provides methods of treating abnormal conditions such as, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease, related to both an excess of and insufficient amounts of TR6 activity.

If the activity of TR6 is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of ligands to the TR6, or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of TR6 polypeptides still capable of binding the ligand in competition with endogenous TR6 may be administered. Typical embodiments of such competitors comprise fragments of the TR6 polypeptide.

In still another approach, expression of the gene encoding endogenous TR6 can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al.*, *Nucleic Acids Res* (1979) 6:3073; Cooney *et al.*, *Science* (1988) 241:456; Dervan *et al.*, *Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of TR6 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates TR6, i.e., an agonist as

described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of TR6 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996). Another approach is to administer a therapeutic amount of TR6 polypeptides in combination with a suitable pharmaceutical carrier.

Formulation and Administration

Peptides, such as the soluble form of TR6 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or

fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 μg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Examples

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples illustrate, but do not limit the invention.

Example 1

Two ESTs (EST#1760054 and EST#1635744) with sequence similarity to the human TNF receptor were discovered in a commercial EST database. Analysis of the two nucleotide sequences (3,466 bp and 2,641 bp respectively), revealed each was a partial sequence of the complete cDNA sequence, overlapping, with 100% identity, 2,226 bp at the nucleotide level. Together, the two sequences encompassed the complete predicted cDNA sequence of 3,881 bp, and encoded an open reading frame for a novel member of the TNF receptor superfamily and named TR6. The predicted protein is 411 amino acids long with a hydrophobic membrane spanning region indicating that at least one form of TR6 is expressed as a membrane bound protein. Comparison of TR6 protein sequence, with other TNF receptor family proteins indicates that it has two of the cysteine-rich repeats characteristic of the extracellular domains of this family, and an intracellular death domain.

Northern blot of TR6.

Various tissues and cell lines were screened for mRNA expression by Northern blot. RNA was prepared from cells and cell lines using Tri-Reagent (Molecular Research Center Inc.; Cincinnati, OH), run in denaturing agarose gels (Sambrook et al., Molecular Cloning: a laboratory manual, 2nd Ed. Cold Spring Harbor Lab Press, NY (1989)) and transfered to Zeta-probe nylon membrane (Biorad, Hercules, CA.) via vacuum blotting in 25mM NaOh for 90 min. After neutralization for 5-10 minutes with 1M tris-HCl, pH 7.5 containing 3M NaCl, the blots were prehybridized with 50% formamide, 8% dextran sulfate, 6XSSPE, 0.1%SDS and 100mg/ml of sheared and denatured salmon sperm DNA for at least 30 min. At 42°C. cDNA probes were labeled with 32P-CTP by random priming (Statagene, La Jolla, CA), briefly denatured with 0.25M NaOH and added to the prehybridization solution. After a further incubation for at least 24h at 42°C, the blots were washed in high stringency conditions and exposed to X-ray film.

Very high expression of TR6 RNA was detected in aortic endothelial cells. High expression was also detected in monocytes. Low expression was detected in bone marrow and

CD4+ activated PBLs. Very low, but detectable levels of TR6 RNA was expressed in CD19+ PBLs, CD8+ PBLs (both activated and unstimulated), and unstimulated CD4+ PBLs.

In hematopoietic cell lines, low levels of TR6 RNA was expressed in HL60 (promyelocyte), KG1a (promyeloblast) and KG1 (myeloblast) cell lines. Very low but detectable levels of TR6 RNA was expressed in U937 (monoblast) and THP-1 (monocyte) cell lines.

The major RNA form is 3.8 kb in size.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: DEEN, KEITH C
YOUNG, PETER R

(ii) TITLE OF THE INVENTION: TUMOR NECROSIS FACTOR RELATED RECEPTOR, TR6

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: RATNER & PRESTIA
(B) STREET: P.O. BOX 980
(C) CITY: VALLEY FORGE
(D) STATE: PA
(E) COUNTRY: USA
(F) ZIP: 19482

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: TO BE ASSIGNED
(B) FILING DATE: 22-AUGUST-1997
(C) CLASSIFICATION: Unknown

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/853,684
(B) FILING DATE: 09-MAY-1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: PRESTIA, PAUL F.
(B) REGISTRATION NUMBER: 23,031
(C) REFERENCE/DOCKET NUMBER: GH-50008-1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 610-407-0700
(B) TELEFAX: 610-407-0701
(C) TELEX: 846169

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3,881 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CTTTGCGCCC ACAAAATACA CCGACGATGC CCGATCTACT TTAAGGGCTG AAACCCACGG 60
GCCTGAGAGA CTATAAGAGC GTTCCCTACC GCCATGGAAC AACGGGGACA GAACGCCCCG 120
GCCGCTTCGG GGGCCCCGAA AAGGCACGGC CCAGGACCCA GGGAGGCGCG GGGAGCCAGG 180
CCTGGGCCEC GGGTCCCCAA GACCCTTGTG CTCGTTGTCG CCGCGGTCCT GCTGTTGGTC 240
TCAGCTGAGT CTGCTCTGAT CACCCAACAA GACCTAGCTC CCCAGCAGAG AGCGGCCCCA 300
CAACAAAAGA GGTCCAGCCC CTCAGAGGGA TTGTGTCCAC CTGGACACCA TATCTCAGAA 360
GACGGTAGAG ATTGCATCTC CTGCAAATAT GGACAGGACT ATAGCACTCA ATGGAATGAC 420
CTCCTTTTGT GCTTGCGCTG CACCAGGTGT GATTCAGGTG AAGTGGAGCT AAGTCCCTGC 480
ACCACGAGCA GAAACACAGT GTGTCAGTGC GAAGAAGGCA CCTTCCGGGA AGAAGATTCT 540
CCTGAGATGT GCCGGAAGTG CCGCACAGGG TGTCCCAGAG GGATGGTCAA GGTCGGTGAT 600
TGTACACCCT GGAGTGACAT CGAATGTGTC CACAAAGAAT CAGGCATCAT CATAGGAGTC 660
ACAGTTGCAG CCGTAGTCTT GATTGTGGCT GTGTTTGTTT GCAAGTCTTT ACTGTGGAAG 720
AAAGTCCTTC CTTACCTGAA AGGCATCTGC TCAGGTGGTG GTGGGGACCC TGAGCGTGTG 780

GACAGAAGCT CACAACGACC TGGGGCTGAG GACAATGTCC TCAATGAGAT CGTGAGTATC 840
TTGCAGCCCA CCCAGGTCCC TGAGCAGGAA ATGGAAGTCC AGGAGCCAGC AGAGCCAACA 900
GGTGTCAACA TGTTGTCCCC CGGGGAGTCA GAGCATCTGC TGGAACCGGC AGAAGCTGAA 960
AGGTCTCAGA GGAGGAGGCT GCTGGTTCCA GCAAATGAAG GTGATCCCAC TGAGACTCTG 1020
AGACAGTGCT TCGATGACTT TGCAGACTTG GTGCCCTTTG ACTCCTGGGA GCCGCTCATG 1080
AGGAAGTTGG GCCTCATGGA CAATGAGATA AAGGTGGCTA AAGCTGAGGC AGCGGGCCAC 1140
AGGGACACCT TGTACACGAT GCTGATAAAG TGGGTCAACA AAACCGGGCG AGATGCCTCT 1200
GTCCACACCC TGCTGGATGC CTTGGAGACG CTGGGAGAGA GACTTGCCAA GCAGAAGATT 1260
GAGGACCACT TGTTGAGCTC TGGAAAGTTC ATGTATCTAG AAGGTAATGC AGACTCTGCC 1320
ATGTCCTAAG TGTGATTCTC TTCAGGAAGT CAGACCTTCC CTGGTTTACC TTTTTTCTGG 1380
AAAAAGCCCA ACTGGACTCC AGTCAGTAGG AAAGTGCCAC AATTGTCACA TGACCGGTAC 1440
TGGAAGAAAC TCTCCCATCC AACATCACCC AGTGGATGGA AGATCCTGTA ACTTTTCACT 1500
GCACTTGGCA TTATTTTTAT AAGCTGAATG TGATAATAAG GACACTATGG AAATGTCTGG 1560
ATCATTCCGT TTGTGCGTAC TTTGAGATTT GGTTTGGGAT GTCATTGTTT TCACAGCACT 1620
TTTTTATCCT AATGTAAATG CTTTATTTAT TTATTTGGGC TACATTGTAA GATCCATCTA 1680
CACAGTCGTT GTCCGACTTC ACTTGATACT ATATGATATG AACCTTTTTT GGGTGGGGGG 1740
TGCGGGGCAG TTCACTCTGT CTCCCAGGCT GGAGTGCAAT GGTGCAATCT TGGCTCACTA 1800
TAGCCTTGAC CTCTCAGGCT CAAGCGATTC TCCCACCTCA GCCATCCAAA TAGCTGGGAC 1860
CACAGGTGTG CACCACCACG CCCGGCTAAT TTTTTGTATT TTGTCTAGAT ATAGGGGCTC 1920
TCTATGTTGC TCAGGGTGGT CTCGAATTCC TGGACTCAAG CAGTCTGCCC ACCTCAGACT 1980
CCCAAAGCGG TGGAATTAGA GGCGTGAGCC CCCATGCTTG GCCTTACCTT TCTACTTTTA 2040
TAATTCTGTA TGTTATTATT TTATGAACAT GAAGAAACTT TAGTAAATGT ACTTGTTTAC 2100
ATAGTTATGT GAATAGATTA GATAAACATA AAAGGAGGAG ACATACAATG GGGGAAGAAG 2160
AAGAAGTCCC CTGTAAGATG TCACTGTCTG GGTTCCAGCC CTCCCTCAGA TGTACTTTGG 2220
CTTCAATGAT TGGCAACTTC TACAGGGGCC AGTCTTTTGA ACTGGACAAC CTTACAAGTA 2280
TATGAGTATT ATTTATAGGT AGTTGTTTAC ATATGAGTCG GGACCAAAGA GAACTGGATC 2340
CACGTGAAGT CCTGTGTGTG GCTGGTCCCT ACCTGGGCAG TCTCATTTGC ACCCATAGCC 2400
CCCATCTATG GACAGGCTGG GACAGAGGCA GATGGGTTAG ATCACACATA ACAATAGGGT 2460
CTATGTCATA TCCCAAGTGA ACTTGAGCCC TGTTTGGGCT CAGGAGATAG AAGACAAAAT 2520
CTGTCTCCCC ACGTCTGCCA TGGCATCAAG GGGGAAGAGT AGATGGTGCT TGAGAATGGT 2580
GTGAAATGGT TGCCATCTCA GGAGTAGATG GCCCGGCTCA CTTCTGGTTA TCTGTCACCC 2640
TGAGCCCATG AGCTGCCTTT TAGGGTACAG ATTGCCTACT TGAGGACCTT GGCCGCTCTG 2700
TAAGCATCTG ACTCATCTCA GAAATGTCAA TTCTTAAACA CTGTGGCAAC AGGACCTAGA 2760
ATGGCTGACG CATTAAGGTT TTCTTCTTGT GTCCTGTTCT ATTATTGTTT TAAGACCTCA 2820
GTAACCATTT CAGCCTCTTT CCAGCAAACC CTTCTCCATA GTATTTCAGT CATGGAAGGA 2880
TCATTTATGC AGGTAGTCAT TCCAGGAGTT TTTGGTCTTT TCTGTCTCAA GGCATTGTGT 2940

```
GTTTTGTTCC GGGACTGGTT TGGGTGGGAC AAAGTTAGAA TTGCCTGAAG ATCACACATT   3000
CAGACTGTTG TGTCTGTGGA GTTTTAGGAG TGGGGGGTGA CCTTTCTGGT CTTTGCACTT   3060
CCATCCTCTC CCACTTCCAT CTGGCATCCC CACGCGTTGT CCCCTGCACT TCTGGAAGGC   3120
ACAGGGTGCT GCTGCTTCCT GGTCTTTGCC TTTGCTGGGC CTTCTGTGCA GGACGCTCAG   3180
CCTCAGGGCT CAGAAGGTGC CAGTCCGGTC CCAGGTCCCT TGTCCCTTCC ACAGAGGCCT   3240
TCCTAGAAGA TGCATCTAGA GTGTCAGCCT TATCAGTGTT TAAGATTTTT CTTTTATTAT   3300
TAATTTTTTT GAGACAGAAT CTCACTCTCT CGCCCAGGCT GGAGTGCAAC GGTACGATCT   3360
TGGCTCAGTG CAACCTCCGC CTCCTGGGTT CAAGCGATTC TCGTGCCTCA GCCTCCGGAG   3420
TAGCTGGGAT TGCAGGCACC CGCCACCACG CCTGGCTAAT TTTTGTATTT TTAGTAGAGA   3480
CGGGGTTTCA CCATGTTGGT CAGGCTGGTC TCGAACTCCT GACCTCAGGT GATCCACNTT   3540
GGCCTCCGAA AGTGCTGGGA TATACAAGGC GTGAGCCACC AGCCAGGCCA AGATATTNTT   3600
NTAAAGNNAG CTTCCGGANG ACATGAAATA ANGGGGGGTT TTGTTGTTTA GTAACATTNG   3660
GCTTTGATAT ATCCCCAGGC CAAATNGCAN GNGACACAGG ACAGCCATAG TATAGTGTGT   3720
CACTCGTGGT TGGTGTCCTT TCATGGTTCT GCCCTGTCAA AGGTCCCTAT TTGAAATGTG   3780
TTATAATACA AACAAGGAAG CACATTGTGT ACAAAATACT TATGTATTTA TGAATCCATG   3840
ACCAAATTAA ATATGAAACC TTATATAAAA AAAAAAAAAA A                       3881
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 411 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Glu Gln Arg Gly Gln Asn Ala Pro Ala Ala Ser Gly Ala Arg Lys
1               5                   10                  15
Arg His Gly Pro Gly Pro Arg Glu Ala Arg Gly Ala Arg Pro Gly Pro
            20                  25                  30
Arg Val Pro Lys Thr Leu Val Leu Val Val Ala Ala Val Leu Leu Leu
        35                  40                  45
Val Ser Ala Glu Ser Ala Leu Ile Thr Gln Gln Asp Leu Ala Pro Gln
    50                  55                  60
```

```
Gln Arg Ala Ala Pro Gln Gln Lys Arg Ser Ser Pro Ser Glu Gly Leu
 65                  70                  75                  80
Cys Pro Pro Gly His His Ile Ser Glu Asp Gly Arg Asp Cys Ile Ser
                 85                  90                  95
Cys Lys Tyr Gly Gln Asp Tyr Ser Thr Gln Trp Asn Asp Leu Leu Phe
            100                 105                 110
Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val Glu Leu Ser Pro
        115                 120                 125
Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu Glu Gly Thr Phe
    130                 135                 140
Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys Arg Thr Gly Cys
145                 150                 155                 160
Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile
                165                 170                 175
Glu Cys Val His Lys Glu Ser Gly Ile Ile Ile Gly Val Thr Val Ala
            180                 185                 190
Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp
        195                 200                 205
Lys Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly
    210                 215                 220
Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp
225                 230                 235                 240
Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val Pro
                245                 250                 255
Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly Val Asn
            260                 265                 270
Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala Glu Ala
        275                 280                 285
Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala Asn Glu Gly Asp
    290                 295                 300
Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe Ala Asp Leu Val
305                 310                 315                 320
Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu Gly Leu Met Asp
                325                 330                 335
Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly His Arg Asp Thr
            340                 345                 350
Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr Gly Arg Asp Ala
        355                 360                 365
Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu Gly Glu Arg Leu
    370                 375                 380
Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser Gly Lys Phe Met
385                 390                 395                 400
```

Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser End
405 410 411

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1062 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGACCTCCT TTTCTGCTTG CGCTGCACCA GGTGTGATTC AGGTGAAGTG GAGCTAAGTC 60
CCTGCACCAC GACCAGAAAC ACAGTGTGTC AGTGCGAAGA AGGCACCTTC CGGGAAGAAG 120
ATTCTCCTGA GATGTGCCGG AAGTGCCGCA CAGGGTGTCC CAGAGGGATG GTCAAGGTCG 180
GTGATTGTAC ACCCTGGAGT GACATCGAAT GTGTCCACAA AGAATCAGGC ATCATCATAG 240
GAGTCACAGT TGCAGCCGTA GTCTTGATTG TGGCTGTGTT TGTTTGCAAG TCTTTACTGT 300
GGAAGAAAGT CCTTCCTTAC CTGAAAGGCA TGTGCTCAGG TGGTGGTGGG GACCCTGAGC 360
GTGTGGACAG AAGCTCACAA CGACCTGGGG CTGAGGACAA TGTCCTCAAT GAGATCGTGA 420
GTATCTTGCA GCCCACCCAG GTCCCTGAGC AGGAAATGGA AGTCCAGGAG CCAGCAGAGC 480
CAACAGGTGT CAACATGTTG TCCCCCGGGG AGTCAGAGCA TCTGCTGGAA CCGGCAGAAG 540
CTGAAAGGTC TCAGAGGAGG AGGCTGCTGG TTCCAGCAAA TGAAGGTGAT CCCACTGAGA 600
CTCTGAGACA GTGCTTCGAT GACTTTGCAG ACTTGGTGCC CTTTGACTCC TGGGAGCCGC 660
TCATGAGGAA GTTGGGCCTC ATGGACAATG AGATAAAGGT GGCTAAAGCT GAGGCAGCGG 720
GCCACAGGGA CACCTTGTAC ACGATGCTGA TAAAGTGGGT CAACAAAACC GGGCGAGATG 780
CCTCTGTCCA CACCCTGCTG GATGCCTTGG AGACGCTGGG AGAGGACTTT GCCAAGCAGA 840
AGATTGAGGA CCACTTGTTG AGCTCTGGAA AGTTCATGTA TCTAGAAGGT AATGCAGACT 900
CTGCCATGTC CTAAGTGTGA TTCTCTTCAG GAAGTCAGAC CTTCCCTGGT TTACCTTTTT 960
TCTGGAAAAA GCCCAACTGG ACTCCAGTCA GTAGGAAAGT GCCACAATTG TCACATGACC 1020
GGTACTGGAA GAGACTCTCC CATCCAACAT CACCCAGTGG AT 1062

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 303 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Asp Leu Leu Phe Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val
 1               5                  10                  15
Glu Leu Ser Pro Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu
            20                  25                  30
Glu Gly Thr Phe Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys
        35                  40                  45
Arg Thr Gly Cys Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro
    50                  55                  60
Trp Ser Asp Ile Glu Cys Val His Lys Glu Ser Gly Ile Ile Ile Gly
65                  70                  75                  80
Val Thr Val Ala Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys
                85                  90                  95
Ser Leu Leu Trp Lys Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser
            100                 105                 110
Gly Gly Gly Gly Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro
        115                 120                 125
Gly Ala Glu Asp Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro
    130                 135                 140
Thr Gln Val Pro Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro
145                 150                 155                 160
Thr Gly Val Asn Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu
                165                 170                 175
Pro Ala Glu Ala Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala
            180                 185                 190
Asn Glu Gly Asp Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe
        195                 200                 205
Ala Asp Leu Val Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu
    210                 215                 220
Gly Leu Met Asp Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly
225                 230                 235                 240
His Arg Asp Thr Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr
                245                 250                 255
Gly Arg Asp Ala Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu
            260                 265                 270
Gly Glu Arg Leu Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser
        275                 280                 285
```

Gly Lys Phe Met Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser
290 295 300

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1769 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCTCACTGAC TATAAAAGAA TAGAGAAGGA AGGGCTTCAG TGACCGGCTG CCTGGCTGAC 60
TTACAGCAGT CAGACTCTGA CAGGATCATG GCTATGATGG AGGTCCAGGG GGGACCCAGC 120
CTGGGACAGA CCTGCGTGCT GATCGTGATC TTCACAGTGC TCCTGCAGTC TCTCTGTGTG 180
GCTGTAACTT ACGTGTACTT TACCAACGAG CTGAAGCAGA TGCAGGACAA GTACTCCAAA 240
AGTGGCATTG CTTGTTTCTT AAAAGAAGAT GACAGTTATT GGGACCCCAA TGACGAAGAG 300
AGTATGAACA GCCCCTGCTG GCAAGTCAAG TGGCAACTCC GTCAGCTCGT TAGAAAGATG 360
ATTTTGAGAA CCTCTGAGGA AACCATTTCT ACAGTTCAAG AAAAGCAACA AAATATTTCT 420
CCCCTAGTGA GAGAAAGAGG TCCTCAGAGA GTAGCAGCTC ACATAACTGG GACCAGAGGA 480
AGAAGCAACA CATTGTCTTC TCCAAACTGC AAGAATGAAA AGGCTCTGGG CCGCAAAATA 540
AACTCCTGGG AATCATCAAG GAGTGGGCAT TCATTCCTGA GCAACTTGCA CTTGAGGAAT 600
GGTGAACTGG TCATCCATGA AAAAGGGTTT TACTACATCT ATTCCCAAAC ATACTTTCGA 660
TTTCAGGAGG AAATAAAAGA AAAGACAAAG AACGACAAAC AAATGGTCCA ATATATTTAC 720
AAATACACAA GTTATCCTGA CCCTATATTG TTGATGAAAA GTGCTAGAAA TAGTTGTTGG 780
TCTAAAGATG CAGAATATGG ACTCTATTCC ATCTATCAAG GGGGAATATT TGAGCTTAAG 840
GAAAATGACA GAATTTTTGT TTCTGTAACA AATGAGCACT TGATAGACAT GGACCATGAA 900
GCCAGTTTTT TCGGGGCGTT TTTAGTTGGC TAACTGACCT GGAAAGAAAA AGCAATAACC 960
TCAAAGTGAC TATTCAGTTT TCAGGATGAT ACACTATGAA GATGTTTCAA AAAATCTGAC 1020
CAAAACAAAC AAACAGAAAA CAGAAAACAA AAAAACCTCT ATGCAATCTG AGTAGAGCAG 1080
CCACAACCAA AAAATTCTAC AACACACACT GTTCTGAAAG TGACTCACTT ATCCCAAGAA 1140
AATGAAATTG GTGAAAGATC TTTCAGGACT CTACCTCATA TCAGTTTGCT AGCAGAAATC 1200
TAGAAGACTG TCAGCTTCCA AACATTAATG CAATGGTTAA CATCTTCTGT CTTTATAATC 1260
TACTCCTTGT AAAGACTGTA GAAGAAAGCG CAACAATCCA TCTCTCAAGT AGTGTATCAC 1320
AGTAGTAGCC TCCAGGTTTC CTTAAGGGAC AACATCCTTA AGTCAAAAGA GAGAAGAGGC 1380
ACCACTAAAA GATCGCAGTT TGCCTGGTGC AGTGGCTCAC ACCTGTAATC CCAACATTTT 1440
GGGAGCCCAA GGTGGGTAGA TCACGAGATC AAGAGATCAA GACCATAGTG ACCAACATAG 1500
TGAAACCCCA TCTCTACTGA AAGTGCAAAA ATTAGCTGGG TGTGTTGGCA CATGCCTGTA 1560
GTCCCAGCTA CTTGAGAGGC TGAGGCAGGA GAATCGTTTG AACCCGGGAG GCAGAGGTTG 1620
CAGTGTGGTG AGATCATGCC ACTACACTCC AGCCTGGCGA CAGAGCGAGA CTTGGTTTCA 1680

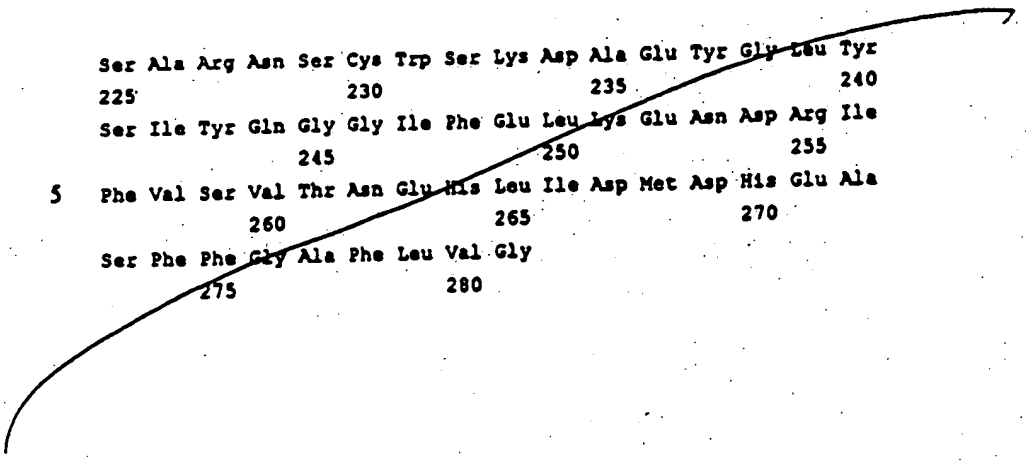
AAAAAAAAAA AAAAAAAAAA CTTCAGTAAG TACGTGTTAT TTTTTTCAAT AAAATTCTAT 1740
TACAGTATGT CAAAAAAAAA AAAAAAAAA 1769

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 281 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ala Met Met Glu Val Gln Gly Gly Pro Ser Leu Gly Gln Thr Cys
1 5 10 15
Val Leu Ile Val Ile Phe Thr Val Leu Leu Gln Ser Leu Cys Val Ala
20 25 30
Val Thr Tyr Val Tyr Phe Thr Asn Glu Leu Lys Gln Met Gln Asp Lys
35 40 45
Tyr Ser Lys Ser Gly Ile Ala Cys Phe Leu Lys Glu Asp Asp Ser Tyr
50 55 60
Trp Asp Pro Asn Asp Glu Glu Ser Met Asn Ser Pro Cys Trp Gln Val
65 70 75 80
Lys Trp Gln Leu Arg Gln Leu Val Arg Lys Met Ile Leu Arg Thr Ser
85 90 95
Glu Glu Thr Ile Ser Thr Val Gln Glu Lys Gln Gln Asn Ile Ser Pro
100 105 110
Leu Val Arg Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile Thr Gly
115 120 125
Thr Arg Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu
130 135 140
Lys Ala Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly
145 150 155 160
His Ser Phe Leu Ser Asn Leu His Leu Arg Asn Gly Glu Leu Val Ile
165 170 175
His Glu Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe
180 185 190
Gln Glu Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val Gln
195 200 205
Tyr Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys
210 215 220



```
Ser Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr
225                 230                 235                 240
Ser Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile
                245                 250                 255
Phe Val Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His Glu Ala
            260                 265                 270
Ser Phe Phe Gly Ala Phe Leu Val Gly
        275                 280
```

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the TR6 polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence.

2. The polynucleotide of claim 1 which is DNA or RNA.

3. The polynucleotide of claim 1 wherein said nucleotide sequence is at least 80% identical to that contained in SEQ ID NO:1.

4. The polynucleotide of claim 3 wherein said nucleotide sequence comprises the TR6 polypeptide encoding sequence contained in SEQ ID NO:1.

5. The polynucleotide of claim 3 which is polynucleotide of SEQ ID NO: 1.

6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing a TR6 polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide of SEQ ID NO:2 when said expression system is present in a compatible host cell.

7. A host cell comprising the expression system of claim 6.

8. A process for producing a TR6 polypeptide comprising culturing a host of claim 7 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture.

9. A process for producing a cell which produces a TR6 polypeptide thereof comprising transforming or transfecting a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces a TR6 polypeptide.

10. A TR6 polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO:2 over its entire length.

11. The polypeptide of claim 10 which comprises the amino acid sequence of SEQ ID NO:2.

12. An antibody immunospecific for the TR6 polypeptide of claim 10.

13. A method for the treatment of a subject in need of enhanced activity or expression of TR6 polypeptide of claim 10 comprising:

(a) administering to the subject a therapeutically effective amount of an agonist to said receptor; and/or

(b) providing to the subject an isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the TR6 polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence in a form so as to effect production of said polypeptide activity *in vivo*.

14. A method for the treatment of a subject having need to inhibit activity or expression of TR6 polypeptide of claim 10 comprising:

(a) administering to the subject a therapeutically effective amount of an antagonist to said receptor; and/or

(b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said receptor; and/or

(c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said receptor for its ligand.

15. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of TR6 polypeptide of claim 10 in a subject comprising:

(a) determining the presence or absence of a mutation in the nucleotide sequence encoding said TR6 polypeptide in the genome of said subject; and/or

(b) analyzing for the presence or amount of the TR6 polypeptide expression in a sample derived from said subject.

16. A method for identifying agonists to TR6 polypeptide of claim 10 comprising:

(a) contacting a cell which produces a TR6 polypeptide with a candidate compound; and

(b) determining whether the candidate compound effects a signal generated by activation of the TR6 polypeptide.

17. An agonist identified by the method of claim 16.

18. The method for identifying antagonists to TR6 polypeptide of claim 10 comprising:

(a) contacting said a cell which produces a TR6 polypeptide with an agonist; and

(b) determining whether the signal generated by said agonist is diminished in the presence of a candidate compound.

19. An antagonist identified by the method of claim 18.

20. A recombinant host cell produced by the process of claim 9 or a membrane thereof expressing a TR6 polypeptide.

ABSTRACT OF THE DISCLOSURE

TR6 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing TR6 polypeptides and polynucleotides in the design of protocols for the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease., among others and diagnostic assays for such conditions.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: DEEN, KEITH C
YOUNG, PETER R

(ii) TITLE OF THE INVENTION: TUMOR NECROSIS FACTOR RELATED RECEPTOR, TR6

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: RATNER & PRESTIA
(B) STREET: P.O. BOX 980
(C) CITY: VALLEY FORGE
(D) STATE: PA
(E) COUNTRY: USA
(F) ZIP: 19482

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: TO BE ASSIGNED
(B) FILING DATE: 22-AUGUST-1997
(C) CLASSIFICATION: Unknown

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/853,684
(B) FILING DATE: 09-MAY-1996

T10370

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: PRESTIA, PAUL F.

(B) REGISTRATION NUMBER: 23,031

(C) REFERENCE/DOCKET NUMBER: GH-50008

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 610-407-0700

(B) TELEFAX: 610-407-0701

(C) TELEX: 846169

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3,881 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CTTTGCGCCC ACAAAATACA CCGACGATGC CCGATCTACT TTAAGGGCTG AAACCCACGG 60
GCCTGAGAGA CTATAAGAGC GTTCCCTACC GCCATGGAAC AACGGGGACA GAACGCCCCG 120
GCCGCTTCGG GGGCCCGGAA AAGGCACGGC CCAGGACCCA GGGAGGCGCG GGGAGCCAGG 180
CCTGGGCCCC GGGTCCCCAA GACCCTTGTG CTCGTTGTCG CCGCGGTCCT GCTGTTGGTC 240
TCAGCTGAGT CTGCTCTGAT CACCCAACAA GACCTAGCTC CCCAGCAGAG AGCGGCCCCA 300
CAACAAAAGA GGTCCAGCCC CTCAGAGGGA TTGTGTCCAC CTGGACACCA TATCTCAGAA 360
GACGGTAGAG ATTGCATCTC CTGCAAATAT GGACAGGACT ATAGCACTCA ATGGAATGAC 420
CTCCTTTTCT GCTTGCGCTG CACCAGGTGT GATTCAGGTG AAGTGGAGCT AAGTCCCTGC 480
ACCACGACCA GAAACACAGT GTGTCAGTGC GAAGAAGGCA CCTTCCGGGA AGAAGATTCT 540
CCTGAGATGT GCCGGAAGTG CCGCACAGGG TGTCCCAGAG GGATGGTCAA GGTCGGTGAT 600
TGTACACCCT GGAGTGACAT CGAATGTGTC CACAAAGAAT CAGGCATCAT CATAGGAGTC 660
ACAGTTGCAG CCGTAGTCTT GATTGTGGCT GTGTTTGTTT GCAAGTCTTT ACTGTGGAAG 720
AAAGTCCTTC CTTACCTGAA AGGCATCTGC TCAGGTGGTG GTGGGGACCC TGAGCGTGTG 780

GACAGAAGCT CACAACGACC TGGGGCTGAG GACAATGTCC TCAATGAGAT CGTGAGTATC 840
TTGCAGCCCA CCCAGGTCCC TGAGCAGGAA ATGGAAGTCC AGGAGCCAGC AGAGCCAACA 900
GGTGTCAACA TGTTGTCCCC CGGGGAGTCA GAGCATCTGC TGGAACCGGC AGAAGCTGAA 960
AGGTCTCAGA GGAGGAGGCT GCTGGTTCCA GCAAATGAAG GTGATCCCAC TGAGACTCTG 1020
AGACAGTGCT TCGATGACTT TGCAGACTTG GTGCCCTTTG ACTCCTGGGA GCCGCTCATG 1080
AGGAAGTTGG GCCTCATGGA CAATGAGATA AAGGTGGCTA AAGCTGAGGC AGCGGGCCAC 1140
AGGGACACCT TGTACACGAT GCTGATAAAG TGGGTCAACA AAACCGGGCG AGATGCCTCT 1200
GTCCACACCC TGCTGGATGC CTTGGAGACG CTGGGAGAGA GACTTGCCAA GCAGAAGATT 1260
GAGGACCACT TGTTGAGCTC TGGAAAGTTC ATGTATCTAG AAGGTAATGC AGACTCTGCC 1320
ATGTCCTAAG TGTGATTCTC TTCAGGAAGT CAGACCTTCC CTGGTTTACC TTTTTTCTGG 1380
AAAAAGCCCA ACTGGACTCC AGTCAGTAGG AAAGTGCCAC AATTGTCACA TGACCGGTAC 1440
TGGAAGAAAC TCTCCCATCC AACATCACCC AGTGGATGGA ACATCCTGTA ACTTTTCACT 1500
GCACTTGGCA TTATTTTTAT AAGCTGAATG TGATAATAAG GACACTATGG AAATGTCTGG 1560
ATCATTCCGT TTGTGCGTAC TTTGAGATTT GGTTTGGGAT GTCATTGTTT TCACAGCACT 1620
TTTTTATCCT AATGTAAATG CTTTATTTAT TTATTTGGGC TACATTGTAA GATCCATCTA 1680
CACAGTCGTT GTCCGACTTC ACTTGATACT ATATGATATG AACCTTTTTT GGGTGGGGGG 1740
TGCGGGGCAG TTCACTCTGT CTCCCAGGCT GGAGTGCAAT GGTGCAATCT TGGCTCACTA 1800
TAGCCTTGAC CTCTCAGGCT CAAGCGATTC TCCCACCTCA GCCATCCAAA TAGCTGGGAC 1860
CACAGGTGTG CACCACCACG CCCGGCTAAT TTTTTGTATT TTGTCTAGAT ATAGGGGCTC 1920
TCTATGTTGC TCAGGGTGGT CTCGAATTCC TGGACTCAAG CAGTCTGCCC ACCTCAGACT 1980
CCCAAAGCGG TGGAATTAGA GGCGTGAGCC CCCATGCTTG GCCTTACCTT TCTACTTTTA 2040
TAATTCTGTA TGTTATTATT TTATGAACAT GAAGAAACTT TAGTAAATGT ACTTGTTTAC 2100
ATAGTTATGT GAATAGATTA GATAAACATA AAAGGAGGAG ACATACAATG GGGGAAGAAG 2160
AAGAAGTCCC CTGTAAGATG TCACTGTCTG GGTTCCAGCC CTCCCTCAGA TGTACTTTGG 2220
CTTCAATGAT TGGCAACTTC TACAGGGGCC AGTCTTTTGA ACTGGACAAC CTTACAAGTA 2280
TATGAGTATT ATTTATAGGT AGTTGTTTAC ATATGAGTCG GGACCAAAGA GAACTGGATC 2340
CACGTGAAGT CCTGTGTGTG GCTGGTCCCT ACCTGGGCAG TCTCATTTGC ACCCATAGCC 2400
CCCATCTATG GACAGGCTGG GACAGAGGCA GATGGGTTAG ATCACACATA ACAATAGGGT 2460
CTATGTCATA TCCCAAGTGA ACTTGAGCCC TGTTTGGGCT CAGGAGATAG AAGACAAAAT 2520
CTGTCTCCCC ACGTCTGCCA TGGCATCAAG GGGGAAGAGT AGATGGTGCT TGAGAATGGT 2580
GTGAAATGGT TGCCATCTCA GGAGTAGATG GCCCGGCTCA CTTCTGGTTA TCTGTCACCC 2640
TGAGCCCATG AGCTGCCTTT TAGGGTACAG ATTGCCTACT TGAGGACCTT GGCCGCTCTG 2700
TAAGCATCTG ACTCATCTCA GAAATGTCAA TTCTTAAACA CTGTGGCAAC AGGACCTAGA 2760
ATGGCTGACG CATTAAGGTT TTCTTCTTGT GTCCTGTTCT ATTATTGTTT TAAGACCTCA 2820
GTAACCATTT CAGCCTCTTT CCAGCAAACC CTTCTCCATA GTATTTCAGT CATGGAAGGA 2880
TCATTTATGC AGGTAGTCAT TCCAGGAGTT TTTGGTCTTT TCTGTCTCAA GGCATTGTGT 2940

GTTTTGTTCC GGGACTGGTT TGGGTGGGAC AAAGTTAGAA TTGCCTGAAG ATCACACATT 3000
CAGACTGTTG TGTCTGTGGA GTTTTAGGAG TGGGGGGTGA CCTTTCTGGT CTTTGCACTT 3060
CCATCCTCTC CCACTTCCAT CTGGCATCCC CACGCGTTGT CCCCTGCACT TCTGGAAGGC 3120
ACAGGGTGCT GCTGCTTCCT GGTCTTTGCC TTTGCTGGGC CTTCTGTGCA GGACGCTCAG 3180
CCTCAGGGCT CAGAAGGTGC CAGTCCGGTC CCAGGTCCCT TGTCCCTTCC ACAGAGGCCT 3240
TCCTAGAAGA TGCATCTAGA GTGTCAGCCT TATCAGTGTT TAAGATTTTT CTTTTATTTT 3300
TAATTTTTTT GAGACAGAAT CTCACTCTCT CGCCCAGGCT GGAGTGCAAC GGTACGATCT 3360
TGGCTCAGTG CAACCTCCGC CTCCTGGGTT CAAGCGATTC TCGTGCCTCA GCCTCCGGAG 3420
TAGCTGGGAT TGCAGGCACC CGCCACCACG CCTGGCTAAT TTTTGTATTT TTAGTAGAGA 3480
CGGGGTTTCA CCATGTTGGT CAGGCTGGTC TCGAACTCCT GACCTCAGGT GATCCACNTT 3540
GGCCTCCGAA AGTGCTGGGA TATACAAGGC GTGAGCCACC AGCCAGGCCA AGATATTNTT 3600
NTAAAGNNAG CTTCCGGANG ACATGAAATA ANGGGGGGTT TTGTTGTTTA GTAACATTNG 3660
GCTTTGATAT ATCCCCAGGC CAAATNGCAN GNGACACAGG ACAGCCATAG TATAGTGTGT 3720
CACTCGTGGT TGGTGTCCTT TCATGGTTCT GCCCTGTCAA AGGTCCCTAT TTGAAATGTG 3780
TTATAATACA AACAAGGAAG CACATTGTGT ACAAAATACT TATGTATTTA TGAATCCATG 3840
ACCAAATTAA ATATGAAACC TTATATAAAA AAAAAAAAAA A 3881

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 411 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Glu Gln Arg Gly Gln Asn Ala Pro Ala Ala Ser Gly Ala Arg Lys
1 5 10 15
Arg His Gly Pro Gly Pro Arg Glu Ala Arg Gly Ala Arg Pro Gly Pro
20 25 30
Arg Val Pro Lys Thr Leu Val Leu Val Val Ala Ala Val Leu Leu Leu
35 40 45
Val Ser Ala Glu Ser Ala Leu Ile Thr Gln Gln Asp Leu Ala Pro Gln
50 55 60
Gln Arg Ala Ala Pro Gln Gln Lys Arg Ser Ser Pro Ser Glu Gly Leu
65 70 75 80

```
Cys Pro Pro Gly His His Ile Ser Glu Asp Gly Arg Asp Cys Ile Ser
                85                  90                  95
Cys Lys Tyr Gly Gln Asp Tyr Ser Thr Gln Trp Asn Asp Leu Leu Phe
            100                 105                 110
Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val Glu Leu Ser Pro
        115                 120                 125
Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu Glu Gly Thr Phe
    130                 135                 140
Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys Arg Thr Gly Cys
145                 150                 155                 160
Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile
                165                 170                 175
Glu Cys Val His Lys Glu Ser Gly Ile Ile Ile Gly Val Thr Val Ala
            180                 185                 190
Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp
        195                 200                 205
Lys Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly
    210                 215                 220
Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp
225                 230                 235                 240
Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val Pro
                245                 250                 255
Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly Val Asn
            260                 265                 270
Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala Glu Ala
        275                 280                 285
Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala Asn Glu Gly Asp
    290                 295                 300
Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe Ala Asp Leu Val
305                 310                 315                 320
Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu Gly Leu Met Asp
                325                 330                 335
Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly His Arg Asp Thr
            340                 345                 350
Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr Gly Arg Asp Ala
        355                 360                 365
Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu Gly Glu Arg Leu
    370                 375                 380
Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser Gly Lys Phe Met
385                 390                 395                 400
Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser End
                405                 410 411
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1062 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATGACCTCCT TTTCTGCTTG CGCTGCACCA GGTGTGATTC AGGTGAAGTG GAGCTAAGTC     60
CCTGCACCAC GACCAGAAAC ACAGTGTGTC AGTGCGAAGA AGGCACCTTC CGGGAAGAAG    120
ATTCTCCTGA GATGTGCCGG AAGTGCCGCA CAGGGTGTCC CAGAGGGATG GTCAAGGTCG    180
GTGATTGTAC ACCCTGGAGT GACATCGAAT GTGTCCACAA AGAATCAGGC ATCATCATAG    240
GAGTCACAGT TGCAGCCGTA GTCTTGATTG TGGCTGTGTT TGTTTGCAAG TCTTTACTGT    300
GGAAGAAAGT CCTTCCTTAC CTGAAAGGCA TCTGCTCAGG TGGTGGTGGG GACCCTGAGC    360
GTGTGGACAG AAGCTCACAA CGACCTGGGG CTGAGGACAA TGTCCTCAAT GAGATCGTGA    420
GTATCTTGCA GCCCACCCAG GTCCCTGAGC AGGAAATGGA AGTCCAGGAG CCAGCAGAGC    480
CAACAGGTGT CAACATGTTG TCCCCCGGGG AGTCAGAGCA TCTGCTGGAA CCGGCAGAAG    540
CTGAAAGGTC TCAGAGGAGG AGGCTGCTGG TTCCAGCAAA TGAAGGTGAT CCCACTGAGA    600
CTCTGAGACA GTGCTTCGAT GACTTTGCAG ACTTGGTGCC CTTTGACTCC TGGGAGCCGC    660
TCATGAGGAA GTTGGGCCTC ATGGACAATG AGATAAAGGT GGCTAAAGCT GAGGCAGCGG    720
GCCACAGGGA CACCTTGTAC ACGATGCTGA TAAAGTGGGT CAACAAAACC GGGCGAGATG    780
CCTCTGTCCA CACCCTGCTG GATGCCTTGG AGACGCTGGG AGAGAGACTT GCCAAGCAGA    840
AGATTGAGGA CCACTTGTTG AGCTCTGGAA AGTTCATGTA TCTAGAAGGT AATGCAGACT    900
CTGCCATGTC CTAAGTGTGA TTCTCTTCAG GAAGTCAGAC CTTCCCTGGT TTACCTTTTT    960
TCTGGAAAAA GCCCAACTGG ACTCCAGTCA GTAGGAAAGT GCCACAATTG TCACATGACC   1020
GGTACTGGAA GAAACTCTCC CATCCAACAT CACCCAGTGG AT                       1062
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 303 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Asp Leu Leu Phe Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val
 1               5                  10                  15
Glu Leu Ser Pro Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu
            20                  25                  30
Glu Gly Thr Phe Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys
        35                  40                  45
Arg Thr Gly Cys Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro
    50                  55                  60
Trp Ser Asp Ile Glu Cys Val His Lys Glu Ser Gly Ile Ile Ile Gly
65                  70                  75                  80
Val Thr Val Ala Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys
                85                  90                  95
Ser Leu Leu Trp Lys Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser
            100                 105                 110
Gly Gly Gly Gly Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro
        115                 120                 125
Gly Ala Glu Asp Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro
```

```
    130                 135                 140
Thr Gln Val Pro Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro
145                 150                 155                 160
Thr Gly Val Asn Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu
                165                 170                 175
Pro Ala Glu Ala Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala
            180                 185                 190
Asn Glu Gly Asp Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe
        195                 200                 205
Ala Asp Leu Val Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu
    210                 215                 220
Gly Leu Met Asp Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly
225                 230                 235                 240
His Arg Asp Thr Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr
                245                 250                 255
Gly Arg Asp Ala Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu
            260                 265                 270
Gly Glu Arg Leu Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser
        275                 280                 285
Gly Lys Phe Met Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser
    290                 295                 300
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1769 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
CCTCACTGAC TATAAAAGAA TAGAGAAGGA AGGGCTTCAG TGACCGGCTG CCTGGCTGAC     60
TTACAGCAGT CAGACTCTGA CAGGATCATG GCTATGATGG AGGTCCAGGG GGGACCCAGC    120
CTGGGACAGA CCTGCGTGCT GATCGTGATC TTCACAGTGC TCCTGCAGTC TCTCTGTGTG    180
GCTGTAACTT ACGTGTACTT TACCAACGAG CTGAAGCAGA TGCAGGACAA GTACTCCAAA    240
AGTGGCATTG CTTGTTTCTT AAAAGAAGAT GACAGTTATT GGGACCCCAA TGACGAAGAG    300
AGTATGAACA GCCCCTGCTG GCAAGTCAAG TGGCAACTCC GTCAGCTCGT TAGAAAGATG    360
ATTTTGAGAA CCTCTGAGGA AACCATTTCT ACAGTTCAAG AAAAGCAACA AAATATTTCT    420
CCCCTAGTGA GAGAAAGAGG TCCTCAGAGA GTAGCAGCTC ACATAACTGG GACCAGAGGA    480
AGAAGCAACA CATTGTCTTC TCCAAACTCC AAGAATGAAA AGGCTCTGGG CCGCAAAATA    540
AACTCCTGGG AATCATCAAG GAGTGGGCAT TCATTCCTGA GCAACTTGCA CTTGAGGAAT    600
GGTGAACTGG TCATCCATGA AAAAGGGTTT TACTACATCT ATTCCCAAAC ATACTTTCGA    660
TTTCAGGAGG AAATAAAAGA AAACACAAAG AACGACAAAC AAATGGTCCA ATATATTTAC    720
AAATACACAA GTTATCCTGA CCCTATATTG TTGATGAAAA GTGCTAGAAA TAGTTGTTGG    780
TCTAAAGATG CAGAATATGG ACTCTATTCC ATCTATCAAG GGGGAATATT TGAGCTTAAG    840
GAAAATGACA GAATTTTTGT TTCTGTAACA AATGAGCACT TGATAGACAT GGACCATGAA    900
GCCAGTTTTT TCGGGGCCTT TTTAGTTGGC TAACTGACCT GGAAAGAAAA AGCAATAACC    960
TCAAAGTGAC TATTCAGTTT TCAGGATGAT ACACTATGAA GATGTTTCAA AAAATCTGAC   1020
CAAAACAAAC AAACAGAAAA CAGAAAACAA AAAAACCTCT ATGCAATCTG AGTAGAGCAG   1080
CCACAACCAA AAAATTCTAC AACACACACT GTTCTGAAAG TGACTCACTT ATCCCAAGAA   1140
AATGAAATTG CTGAAAGATC TTTCAGGACT CTACCTCATA TCAGTTTGCT AGCAGAAATC   1200
TAGAAGACTG TCAGCTTCCA AACATTAATG CAATGGTTAA CATCTTCTGT CTTTATAATC   1260
TACTCCTTGT AAAGACTGTA GAAGAAAGCG CAACAATCCA TCTCTCAAGT AGTGTATCAC   1320
AGTAGTAGCC TCCAGGTTTC CTTAAGGGAC AACATCCTTA AGTCAAAAGA GAGAAGAGGC   1380
ACCACTAAAA GATCGCAGTT TGCCTGGTGC AGTGGCTCAC ACCTGTAATC CCAACATTTT   1440
GGGAACCCAA GGTGGGTAGA TCACGAGATC AAGAGATCAA GACCATAGTG ACCAACATAG   1500
```

```
TGAAACCCCA TCTCTACTGA AAGTGCAAAA ATTAGCTGGG TGTGTTGGCA CATGCCTGTA   1560
GTCCCAGCTA CTTGAGAGGC TGAGGCAGGA GAATCGTTTG AACCCGGGAG GCAGAGGTTG   1620
CAGTGTGGTG AGATCATGCC ACTACACTCC AGCCTGGCGA CAGAGCGAGA CTTGGTTTCA   1680
AAAAAAAAAA AAAAAAAAAA CTTCAGTAAG TACGTGTTAT TTTTTTCAAT AAAATTCTAT   1740
TACAGTATGT CAAAAAAAAA AAAAAAAAA                                      1769
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 281 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Ala Met Met Glu Val Gln Gly Gly Pro Ser Leu Gly Gln Thr Cys
 1               5                  10                  15
Val Leu Ile Val Ile Phe Thr Val Leu Leu Gln Ser Leu Cys Val Ala
            20                  25                  30
Val Thr Tyr Val Tyr Phe Thr Asn Glu Leu Lys Gln Met Gln Asp Lys
        35                  40                  45
Tyr Ser Lys Ser Gly Ile Ala Cys Phe Leu Lys Glu Asp Asp Ser Tyr
    50                  55                  60
Trp Asp Pro Asn Asp Glu Glu Ser Met Asn Ser Pro Cys Trp Gln Val
65                  70                  75                  80
Lys Trp Gln Leu Arg Gln Leu Val Arg Lys Met Ile Leu Arg Thr Ser
                85                  90                  95
Glu Glu Thr Ile Ser Thr Val Gln Glu Lys Gln Gln Asn Ile Ser Pro
            100                 105                 110
Leu Val Arg Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile Thr Gly
        115                 120                 125
Thr Arg Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu
    130                 135                 140
Lys Ala Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly
145                 150                 155                 160
His Ser Phe Leu Ser Asn Leu His Leu Arg Asn Gly Glu Leu Val Ile
                165                 170                 175
His Glu Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe
            180                 185                 190
Gln Glu Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val Gln
        195                 200                 205
Tyr Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys
    210                 215                 220
Ser Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr
225                 230                 235                 240
Ser Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile
                245                 250                 255
Phe Val Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His Glu Ala
            260                 265                 270
Ser Phe Phe Gly Ala Phe Leu Val Gly
        275                 280
```

GH-50008

Tumor Necrosis Factor Related Receptor, TR6

This application claims the benefit of U.S. Provisional Application No: 60/041,230, filed March 14, 1997.

FIELD OF INVENTION

This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to Tumor Necrosis Factor Related family, hereinafter referred to as TR6. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

BACKGROUND OF THE INVENTION

Many biological actions, for instance, response to certain stimuli and natural biological processes, are controlled by factors, such as cytokines. Many cytokines act through receptors by engaging the receptor and producing an intracellular response.

For example, tumor necrosis factors (TNF) alpha and beta are cytokines which act through TNF receptors to regulate numerous biological processes, including protection against infection and induction of shock and inflammatory disease. The TNF molecules belong to the "TNF-ligand" superfamily, and act together with their receptors or counter-ligands, the "TNF-receptor" superfamily. So far, nine members of the TNF ligand superfamily have been identified and ten members of the TNF-receptor superfamily have been characterized.

Among the ligands there are included TNF-α, lymphotoxin-α (LT-α, also known as TNF-β), LT-β (found in complex heterotrimer LT-α2-β), FasL, CD40L, CD27L, CD30L, 4-1BBL, OX40L and nerve growth factor (NGF)). The superfamily of TNF receptors includes the p55TNF receptor, p75TNF receptor, TNF receptor-related protein, FAS antigen or APO-1, CD40, CD27, CD30, 4-1BB, OX40, low affinity p75 and NGF-receptor (Meager, A., Biologicals, 22:291-295 (1994)).

Many members of the TNF-ligand superfamily are expressed by activated T-cells, implying that they are necessary for T-cell interactions with other cell types which underlie cell

ontogeny and functions. (Meager, A., supra).

Considerable insight into the essential functions of several members of the TNF receptor family has been gained from the identification and creation of mutants that abolish the expression of these proteins. For example, naturally occurring mutations in the FAS antigen and its ligand cause lymphoproliferative disease (Watanabe-Fukunaga, R., et al., Nature 356:314 (1992)), perhaps reflecting a failure of programmed cell death. Mutations of the CD40 ligand cause an X-linked immunodeficiency state characterized by high levels of immunoghubulin M and low levels of immunoglobulin G in plasma, indicating faulty T-cell-dependent B-cell activation (Allen, R.C. et al., Science 259:990 (1993)). Targeted mutations of the low affinity nerve growth factor receptor cause a disorder characterized by faulty sensory innovation of peripheral structures (Lee, K.F. et al, Cell 69:737 (1992)).

TNF and LT-α are capable of binding to two TNF receptors (the 55- and 75-kd TNF receptors). A large number of biological effects elicited by TNF and LT-α, acting through their receptors, include hemorrhagic necrosis of transplanted tumors, cytotoxicity, a role in endotoxic shock, inflammation, immunoregulation, proliferation and anti-viral responses, as well as protection against the deleterious effects of ionizing radiation. TNF and LT-α are involved in the pathogenesis of a wide range of diseases, including endotoxic shock, cerebral malaria, tumors, autoimmuine disease, AIDS and graft-host rejection (Beutler, B. and Von Huffel, C., Science 264:667-668 (1994)). Mutations in the p55 Receptor cause increased susceptibility to microbial infection.

Moreover, an about 80 amino acid domain near the C-terminus of TNFR1 (P55) and Fas was reported as the "death domain," which is responsible for transducing signals for programmed cell death (Tartaglia et al., Cell 74:845 (1993)).

The effects of TNF family ligands and TNF family receptors are varied and influence numerous functions, both normal and abnormal, in the biological processes of the mammalian system. There is a clear need, therefore, for identification and characterization of such receptors and ligands that influence biological activity, both normally and in disease states. In particular, there is a need to isolate and characterize novel members of the TNF receptor family.

This indicates that these receptors have an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further receptors which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to TR6 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such TR6 polypeptides and polynucleotides. Such uses include the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with TR6 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate TR6 activity or levels.

DESCRIPTION OF THE INVENTION

Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"TR6" refers, among others, to a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2, or an allelic variant thereof.

"Receptor Activity" or "Biological Activity of the Receptor" refers to the metabolic or physiologic function of said TR6 including similar activities or improved

activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said TR6.

"TR6 gene" refers to a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO:1 or allelic variants thereof and/or their complements.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein

cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two

polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., *et al.*, *Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. *et al.*, *J Molec Biol* (1990) 215:403).

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO: 1 is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO: 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5 or 3 terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference amino acid sequence of SEQ ID NO:2 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO: 2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may

be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

Polypeptides of the Invention

In one aspect, the present invention relates to TR6 polypeptides. The TR6 polypeptides include the polypeptides of SEQ ID NOS:2 and 4; as well as polypeptides comprising the amino acid sequence of SEQ ID NO:2; and polypeptides comprising the amino acid sequence which have at least 80% identity to that of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Also included within TR6 polypeptides are polypeptides having the amino acid sequence which have at least 80% identity to the polypeptide having the amino acid sequence of SEQ ID NO: 2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Preferably TR6 polypeptides exhibit at least one biological activity of the receptor.

The TR6 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

Fragments of the TR6 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned TR6 polypeptides. As with TR6 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of

polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of TR6 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of TR6 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate receptor activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Preferably, all of these polypeptide fragments retain the biological activity of the receptor, including antigenic activity. Among the most preferred fragment is that having the amino acid sequence of SEQ ID NO: 4. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

The TR6 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

Another aspect of the invention relates to TR6 polynucleotides. TR6 polynucleotides include isolated polynucleotides which encode the TR6 polypeptides and fragments, and polynucleotides closely related thereto. More specifically, TR6 polynucleotide of the invention include a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO:1 encoding a TR6 polypeptide of SEQ ID NO: 2, and polynucleotides having the particular sequences of SEQ ID NOS:1 and 3. TR6 polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the TR6 polypeptide of SEQ ID NO:2 over its entire length, and a polynucleotide that is at least 80% identical to that having SEQ ID NO:1 over its entire length. In this regard, polynucleotides at least 90% identical are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under TR6 polynucleotides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such TR6 polynucleotides.

TR6 of the invention is structurally related to other proteins of the Tumor Necrosis Factor Related family, as shown by the results of sequencing the cDNA encoding human TR6. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide numbers 94 to 1329) encoding a polypeptide of 411 amino acids of SEQ ID NO:2. The amino acid sequence of Table 1 (SEQ ID NO:2) has about 58% identity (using GAP (From GCG)) in 411 amino acid residues with DR4, the receptor for the ligand TRAIL. (Pan,G., O'Rourke,K., Chinnaiyan,A.M., Gentz,R., Ebner,R., Ni,J. and Dixit,V.M., Science 276, 111-113 (1997)). The nucleotide sequence of Table 1 (SEQ ID NO:1) has about 70% identity (using GAP (from GCG)) in 1335 nucleotide residues with DR4, the receptor for the ligand TRAIL. TR6 contains a death domain (amino acids 290 to 324 in SEQ ID NO:2) which is 64% identical to the death domain of the human Death receptor 4 (DR4) (Pan,G., O'Rourke,K.,

Chinnaiyan,A.M., Gentz,R., Ebner,R., Ni,J. and Dixit,V.M., Science 276, 111-113 (1997)), 35.7% identical to the death domain of the human Death receptor 3 (DR3) (A.M. Chinnaiyan, et al, Science 274 (5289), 990-992 (1996)), 32.7% identical to the death domain of human TNFR-1, and 19.6% identical to the death domain of CD95 (Fas) (I. Cascino, J. Immunol. 154 (6), 2706-2713 (1995)).

Table 1*

```
   1  CTTTGCGCCC ACAAAATACA CCGACGATGC CCGATCTACT TTAAGGGCTG
  51  AAACCCACGG GCCTGAGAGA CTATAAGAGC GTTCCCTACC GCCATGGAAC
 101  AACGGGGACA GAACGCCCCG GCCGCTTCGG GGGCCCGGAA AAGGCACGGC
 151  CCAGGACCCA GGGAGGCGCG GGGAGCCAGG CCTGGGCCCC GGGTCCCCAA
 201  GACCCTTGTG CTCGTTGTCG CCGCGGTCCT GCTGTTGGTC TCAGCTGAGT
 251  CTGCTCTGAT CACCCAACAA GACCTAGCTC CCCAGCAGAG AGCGGCCCCA
 301  CAACAAAAGA GGTCCAGCCC CTCAGAGGGA TTGTGTCCAc cTGGACACCA
 351  TATCTCAGAA GACGGTAGAG ATTGCATCTC CTGCAAATAT gGACAGGACT
 401  ATAGCACTCA aTGGAATGAC CTCCTTTTCT GCTTGCGCTG CACCAGGTGT
 451  GATTCAGGTG AAGTGGAGCT AAGTCCCTGC ACCACGACCA GAAACACAGT
 501  GTGTCAGTGC GAAGAAgGCA CCTTCCGGGA AGAAGATTCT CCTGAGATGT
 551  GCCGGAAGTG CCGCACAGGG TGTCCCAgAG GGATGGTCAA GGTCGGTGAT
 601  TGTACACCCT GGAGTGACAT CGAATGTGTC CACAAAGAAT CAGGCATCAT
 651  CATAgGAGTC ACAGTTGCAG CCGTAGTCTT GATTGTGGCT GTGTTTGTTT
 701  GCaAgTCTTT ACTGTGGAAg AAAGTCCTTC CTTACCTGAA AGGCATCTGC
 751  TCAGGTGGTG GTGGGGACCC TGAGCGTGTG GACAGAAGcT CACAACGACc
```

```
801  TGGGGCTGAG GACAATGTCC TCAATGAGAT CGTGAGTATC TTGCAGCCCA
851  CCCAGGTCCC TGAGCAGGAA ATGGAAGTCC AGGAGCCAGC AGAGCCAACA
901  GGTGTCAACA TGTTGTCCCC CGGGGAGTCA GAGCATCTGC TGGAACCGGC
951  AGAAGCTGAA AGGTCTCAGA GGAGGAGGCT GCTGGTTCCA GCAAATGAAG
1001 GTGATCCCAC TGAGACTCTG AGACAGTGCT TCGATGACTT TGCAGACTTG
1051 GTGCCCTTTG ACTCCTGGGA gCCgCTCATG AGGAAGTTGG GCCTCATGGA
1101 CAATgAGATa aaGGTGGCTA AAGCTGAGGC AGCGGGCCAC AGGGACACCT
1151 TGTACACGAT GCTGATAAAG TGGGTCAACA AAACCGGGCG AGATGCCTCT
1201 GTCCACACCC TGCTGGATGC CTTGGAGACG CTGGGAGAGA GACTTGCCAA
1251 GCAGAAGATT GAGGACCACT TGTTGAGCTC TGGAAAGTTC ATGTATCTAG
1301 AAGGTAATGC AGACTCTGCC ATGTCCTAAG TGTGATTCTC TTCAGGAAGT
1351 CAGACCTTCC CTGGTTTACC TTTTTTCTGG AAAAAGCCCA ACTGGACTCC
1401 AGTCAGTAGG AAAGTGCCAC AATTGTCACA TGACCGGTAC TGGAAGAAAC
1451 TCTCCCATCC AACATCACCC AGTGGATGGA ACATCCTGTA ACTTTTCACT
1501 GCACTTGGCA TTATTTTTAT AAGCTGAATG TGATAATAAG GACACTATGG
1551 AAATGTCTGG ATCATTCCGT TTGTGCGTAC TTTGAgATTT GGTTTGGGAT
1601 GTCATTGTTT TCACAGCACT TTTTTATCCT AATGTAAATG CTTTATTTAT
1651 TTATTTGGGC TACATTGTAA gATCCATCTA CACAGTCGTT GTCCGACTTC
1701 ACTTGATACT ATATGATATG AACCTTTTTT GGGTGGGGGG TGCGGGGCAg
1751 TTCACTCTGT CTCCCAGGCT GGAGTGCAAT GGTGCAATCT TGGCTCACTA
1801 TAGCCTTGAC CTCTCAGGCT CAAGCGATTC TCCCACCTCA GCCATCCAAA
```

```
1851  TAGCTGGGAC CACAGGTGTG CACCACCACG CCCGGCTAAT TTTTTGTATT
1901  TTGTCTAgAT ATAGGGGCTC TCTATGTTGC TCAGGGTGGT CTCgAATTCC
1951  TGGAcTCAAG CAGTCTGCCC ACcTCAGAcT CCCAAAGCGG TGGAATTAGA
2001  GGCGTGAGCC CCCATGcTTG gCCTTACcTT TcTACTTTTA TAATTCTGTA
2051  TGTTATTATT TTATGAACAT GAAGAAACTT TAGTAAATGT ACTTGTTTAC
2101  ATAGTTATGT GAATAGATTA GATAAACATA AAAGGAGGAG ACATACAATG
2151  GGGGAAGAAG AAGAAGTCCC CTGTAAGATG TCACTGTcTG GGTTCCAGCC
2201  CTCCCTCAGA TGTACTTTGG CTTCAATGAT TGGCAACTTC TACAGGGGCC
2251  AGTCTTTTGA ACTGGACAAC CTTACAAGTA TATGAGTATT ATTTATAGGT
2301  AGTTGTTTAC ATATGAGTCG GGACCAAAGA GAACTGGATC CACGTGAAGT
2351  CCTGTGTGTG GCTGGTCCCT ACCTGGGCAG TcTCATTTGC ACCCATAGCC
2401  CCCATCTATG GACAGGCTGG GACAGAGGCA GATGGGTTAG ATCACACATA
2451  ACAATAGGGT CTATGTCATA TCCCAAGTGA ACTTGAGCCC TGTTTGGGCT
2501  CAGGAGATAG AAGACAAAAT CTGTCTCCCC ACGTCTGCCA TGGCATCAAG
2551  GGGGAAGAGT AGATGGTGCT tGAGAATGGT GTGAAATGGT TGCCATCTCA
2601  GGAGTAGATG GCCCGGCTCA CTTCTGGTTA TCtGTCACCC TGAGCCCAtG
2651  AGCTGCcTTT TAGGGTACAG ATTGCCTACT TGAGGACCTT GGCCGCTCTG
2701  TAAGCATCTG ACTCATCTCA GAAATGTCAA TTCTTAAACA CTGTGGCAAC
2751  AGGACCTAGA ATGGCTGACG CATTAAGGTT TTCTTcTTGT GTCCTGTTCT
2801  ATTAtTGTTT TAAGACCTCA GTAACCATTT CAGCCTCTTT CCAGCAAACC
```

```
2851  CTTCTCCATA GTATTTCAGT CATGGAAGGA TCATTTATGC AGGTAGTCAT

2901  TCCAGGAGTT TTTGGTCTTT TCTGTCTCAA GGCATTGTGT GTTTTGTTCC

2951  GGGACTGGTT TGGGTGGGAC AAAGTTAGAA TTGCCTGAAG ATcAcACATT

3001  CAGACTGTtG TGTCTGTGGA GTTTTAGGAG TGGGGGGTGA CCTTTcTGGT

3051  CTTtGcAcTT CCATCcTcTC CCAcTTCCAT cTGGCATCCC CACGcGTTGT

3101  CCCcTGCAcT TcTGGAAGGC ACAGGGTGCT GCTGCTTCCT GGTCTTTGCC

3151  TTTGCTGGGC cTTCTGTGCA GGACGCTCAG CCTCAGGGCT CAGAAGGTGC

3201  CAGTCCGGTC CCAGGTCCCT TGTCCCTTCC ACAGAGGCCT TCcTAGAAGA

3251  TGCATCTAGA GTGTCAGCCT TATCAGTGTT TAAGATTTTT CTTTTATTTT

3301  TAATTTTTTT GAGACAGAAT CTCACTCTCT CGCCCAGGCT GGAGTGCAAC

3351  GGTACGATCT TGGCTCAGTG CAACCTCCGC CTCCTGGGTT CAAGCGATTC

3401  TCGTGCCTCA GCCTCCGGAG TAGCTGGGAT TGCAGGCACC CGCCACCACG

3451  CCTGGCTAAT TTTTGTATTT TTAGTAGAGA CGGGGTTTCA CCATGTTGGT

3501  CAGGCTGGTC TCGAACTCCT GACCTCAGGT GATCCACNTT GGCCTCCGAA

3551  AGTGCTGGGa tatacaaggc GTGAGCCACC AGCCAGGCCA AGATATTNTT

3601  NTAAAGNNAG CTTCCGGANG ACATGAAATA ANGGGGGGTT TTGTTGTTTA

3651  GTAACATTNG GCTTTGATAT ATCCCCAGGC CAAATNGCAN GNGACACAGG

3701  ACAGCCATAG TATAGTGTGT CACTCGTGGT TGGTGTCCTT TCATGGTTcT

3751  GCCCTGTCAA AGGTCCCTAT TTGAAATGTG TTATAATACA AACAAGGAAG

3801  CACATTGTGT ACAAAATACT TATGTATTTA TGAATCCATG ACCAAATTAA

3851  ATATGAAACC TTATATAAAA AAAAAAAAAA A
```

[redacted]

[a] A nucleotide sequence of a human TR6. (SEQ ID NO: 1).

Table 2[b]

1	Met Glu Gln Arg Gly Gln Asn Ala Pro Ala Ala Ser Gly Ala Arg Lys	16
17	Arg His Gly Pro Gly Pro Arg Glu Ala Arg Gly Ala Arg Pro Gly Pro	32
33	Arg Val Pro Lys Thr Leu Val Leu Val Val Ala Ala Val Leu Leu Leu	48
49	Val Ser Ala Glu Ser Ala Leu Ile Thr Gln Gln Asp Leu Ala Pro Gln	64
65	Gln Arg Ala Ala Pro Gln Gln Lys Arg Ser Ser Pro Ser Glu Gly Leu	80
81	Cys Pro Pro Gly His His Ile Ser Glu Asp Gly Arg Asp Cys Ile Ser	96
97	Cys Lys Tyr Gly Gln Asp Tyr Ser Thr Gln Trp Asn Asp Leu Leu Phe	112
113	Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val Glu Leu Ser Pro	128
129	Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu Glu Gly Thr Phe	144
145	Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys Arg Thr Gly Cys	160
161	Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile	176
177	Glu Cys Val His Lys Glu Ser Gly Ile Ile Ile Gly Val Thr Val Ala	192
193	Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp	208
209	Lys Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly	224
225	Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp	240
241	Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val Pro	256
257	Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly Val Asn	272
273	Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala Glu Ala	288

289	Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala Asn Glu Gly Asp	304
305	Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe Ala Asp Leu Val	320
321	Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu Gly Leu Met Asp	336
337	Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly His Arg Asp Thr	352
353	Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr Gly Arg Asp Ala	368
369	Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu Gly Glu Arg Leu	384
385	Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser Gly Lys Phe Met	400
401	Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser End	411

An amino acid sequence of a human TR6. (SEQ ID NO: 2).

One polynucleotide of the present invention encoding TR6 may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of human of human thymus stromal cells, monocytes, peripheral blood lymphocytes, primary dendritic, and bone marrow cells using the expressed sequence tag (EST) analysis (Adams, M.D., *et al. Science* (1991) 252:1651-1656; Adams, M.D. *et al., Nature*, (1992) *355*:632-634; Adams, M.D., *et al., Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

The nucleotide sequence encoding TR6 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in Table 1 (nucleotide number 94 to 1329 of SEQ ID NO:1), or it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of TR6 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or

secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further preferred embodiments are polynucleotides encoding TR6 variants comprising the amino acid sequence of TR6 polypeptide of Table 1 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination. Among the preferred polynucleotides of the present invention is contained in Table 3 (SEQ ID NO: 3) encoding the amino acid sequence of Table 4 (SEQ ID NO: 4).

Table 3*

```
   1  ATGACCTCCT TTTCTGCTTG CGCTGCACCA GGTGTGATTC AGGTGAAGTG

  51  GAGCTAAGTC CCTGCACCAC GACCAGAAAC ACAGTGTGTC AGTGCGAAGA

 101  AgGCACCTTC CGGGAAGAAG ATTCTCCTGA GATGTGCCGG AAGTGCCGCA

 151  CAGGGTGTCC CAgAGGGATG GTCAAGGTCG GTGATTGTAC ACCCTGGAGT

 201  GACATCGAAT GTGTCCACAA AGAATCAGGC ATCATCATAg GAGTCACAGT

 251  TGCAGCCGTA GTCTTGATTG TGGCTGTGTT TGTTTGCaAg TCTTTACTGT

 301  GGAAgAAAGT CCTTCCTTAC CTGAAAGGCA TCTGCTCAGG TGGTGGTGGG

 351  GACCCTGAGC GTGTGGACAG AAGcTCACAA CGACcTGGGG CTGAGGACAA

 401  TGTCCTCAAT GAGATCGTGA GTATCTTGCA GCCCACCCAG GTCCCTGAGC

 451  AGGAAATGGA AGTCCAGGAG CCAGCAGAGC CAACAGGTGT CAACATGTTG

 501  TCCCCCGGGG AGTCAGAGCA TCTGCTGGAA CCGGCAGAAG CTGAAAGGTC
```

```
 551  TCAGAGGAGG AGGCTGCTGG TTCCAGCAAA TGAAGGTGAT CCCACTGAGA
 601  CTCTGAGACA GTGCTTCGAT GACTTTGCAG ACTTGGTGCC CTTTGACTCC
 651  TGGGAgCCgC TCATGAGGAA GTTGGGCCTC ATGGACAATg AGATaaaGGT
 701  GGCTAAAGCT GAGGCAGCGG GCCACAGGGA CACCTTGTAC ACGATGCTGA
 751  TAAAGTGGGT CAACAAAACC GGGCGAGATG CCTCTGTCCA CACCCTGCTG
 801  GATGCCTTGG AGACGCTGGG AGAGAGACTT GCCAAGCAGA AGATTGAGGA
 851  CCACTTGTTG AGCTCTGGAA AGTTCATGTA TCTAGAAGGT AATGCAGACT
 901  CTGCCATGTC CTAAGTGTGA TTCTCTTCAG GAAGTCAGAC CTTCCCTGGT
 951  TTACCTTTTT TCTGGAAAAA GCCCAACTGG ACTCCAGTCA GTAGGAAAGT
1001  GCCACAATTG TCACATGACC GGTACTGGAA GAAACTCTCC CATCCAACAT
1051  CACCCAGTGG AT
```

[4] A partial nucleotide sequence of a human TR6. (SEQ ID NO: 3).

Table 4[4]

```
   1  DLLFCLRCTR CDSGEVELSP CTTTRNTVCQ CEEGTFREED SPEMCRKCRT
  51  GCPRGMVKVG DCTPWSDIEC VHKESGIIIG VTVAAVVLIV AVFVCKSLLW
 101  KKVLPYLKGI CSGGGGDPER VDRSSQRPGA EDNVLNEIVS ILQPTQVPEQ
 151  EMEVQEPAEP TGVNMLSPGE SEHLLEPAEA ERSQRRRLLV PANEGDPTET
 201  LRQCFDDFAD LVPFDSWEPL MRKLGLMDNE IKVAKAEAAG HRDTLYTMLI
 251  KWVNKTGRDA SVHTLLDALE TLGERLAKQK IEDHLLSSGK FMYLEGNADS
 301  AMS*
```

[4] A partial amino acid sequence of a human TR6. (SEQ ID NO: 4).

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences.

Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1 or a fragment thereof, including that of SEQ ID NO:3, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding TR6 and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the TR6 gene. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

In one embodiment, to obtain a polynucleotide encoding TR6 polypeptide comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof, including that of SEQ ID NO: 3, and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to those of skill in the art. Thus in another aspect, TR6 polynucleotides of the present invention further include a nucleotide sequence comprising a nucleotide sequence that hybridize under stringent condition to a nucleotide sequence having SEQ ID NO: 1 or a fragment thereof, including that of SEQ ID NO:3. Also included with TR6 polypeptides are polypeptide comprising amino acid sequence encoded by nucleotide sequence obtained by the above hybridization condition. Stringent hybridization conditions are as defined above or alternatively conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

Vectors, Host Cells, Expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and

bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL* (*supra*).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If the TR6 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If TR6 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

TR6 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention also relates to the use of TR6 polynucleotides for use as diagnostic reagents. Detection of a mutated form of TR6 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of TR6. Individuals carrying mutations in the TR6 gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled TR6 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al.*, *Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al.*, *Proc Natl Acad Sci USA* (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotides probes comprising TR6 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee et al., Science, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease, through detection of mutation in the TR6 gene by the methods described.

In addition, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease, can be diagnosed by methods comprising determining from a sample derived from a

subject an abnormally decreased or increased level of TR6 polypeptide or TR6 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an TR6, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes). The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

The 3' untranslated region of TR6 matches the 295 bp nucleotide sequence of a mapped EST (Genbank ID: D20151). This EST has been mapped by the Whitehead Institute to chromosome 8, 97.68 cR from the top of the Chromosome 8 linkage group

Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the TR6 polypeptides. The term "immunospecific" means that the antibodies have substantiall greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the TR6 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against TR6 polypeptides may also be employed to treat chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease, among others.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with TR6 polypeptide, or

a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease, among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering TR6 polypeptide via a vector directing expression of TR6 polynucleotide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a TR6 polypeptide wherein the composition comprises a TR6 polypeptide or TR6 gene. The vaccine formulation may further comprise a suitable carrier. Since TR6 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation instonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

The TR6 polypeptide of the present invention may be employed in a screening process for compounds which bind the receptor and which activate (agonists) or inhibit activation of

(antagonists) the receptor polypeptide of the present invention. Thus, polypeptides of the invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

TR6 polypeptides are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate TR6 on the one hand and which can inhibit the function of TR6 on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease.

Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease.

In general, such screening procedures involve producing appropriate cells which express the receptor polypeptide of the present invention on the surface thereof. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells expressing the receptor (or cell membrane containing the expressed receptor) are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response.

The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the receptor is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor. Further, these assays may test whether the candidate compound results in a signal generated by activation of the receptor, using detection systems appropriate to the cells bearing the receptor at their surfaces. Inhibitors of activation are

generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Standard methods for conducting such screening assays are well understood in the art.

Examples of potential TR6 antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligand of the TR6, e.g., a fragment of the ligand, or small molecules which bind to the receptor but do not elicit a response, so that the activity of the receptor is prevented.

Prophylactic and Therapeutic Methods

This invention provides methods of treating abnormal conditions such as, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease, related to both an excess of and insufficient amounts of TR6 activity.

If the activity of TR6 is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of ligands to the TR6, or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of TR6 polypeptides still capable of binding the ligand in competition with endogenous TR6 may be administered. Typical embodiments of such competitors comprise fragments of the TR6 polypeptide.

In still another approach, expression of the gene encoding endogenous TR6 can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al.*, *Nucleic Acids Res* (1979) 6:3073; Cooney *et al.*, *Science* (1988)

241:456; Dervan *et al., Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of TR6 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates TR6, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of TR6 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996). Another approach is to administer a therapeutic amount of TR6 polypeptides in combination with a suitable pharmaceutical carrier.

Formulation and Administration

Peptides, such as the soluble form of TR6 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 µg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Examples

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples illustrate, but do not limit the invention.

Example 1

Two ESTs (EST#1760054 and EST#1635744) with sequence similarity to the human TNF receptor were discovered in a commercial EST database. Analysis of the two nucleotide sequences (3,466 bp and 2,641 bp respectively), revealed each was a partial sequence of the complete cDNA sequence, overlapping, with 100% identity, 2,226 bp at the nucleotide level. Together, the two sequences encompassed the complete predicted cDNA sequence of 3,881 bp, and encoded an open reading frame for a novel member of the TNF receptor superfamily and named TR6. The predicted protein is 411 amino acids long with a hydrophobic membrane spanning region indicating that at least one form of TR6 is expressed as a membrane bound protein. Comparison of TR6 protein sequence, with other TNF receptor family proteins indicates that it has two of the cysteine-rich repeats characteristic of the extracellular domains of this family, and an intracellular death domain.

Northern blot of TR6.

Various tissues and cell lines were screened for mRNA expression by Northern blot. RNA was prepared from cells and cell lines using Tri-Reagent (Molecular Research Center Inc., Cincinnati, OH), run in denaturing agarose gels (Sambrook et al., Molecular Cloning: a laboratory manual, 2nd Ed. Cold Spring Harbor Lab Press, NY (1989)) and transfered to Zeta-probe nylon membrane (Biorad, Hercules, CA.) via vacuum blotting in 25mM NaOh for 90 min. After neutralization for 5-10 minutes with 1M tris-HCl, pH 7.5 containing 3M NaCl, the blots were prehybridized with 50% formamide, 8% dextran sulfate, 6XSSPE, 0.1%SDS and 100mg/ml of sheared and dentured salmon sperm DNA for at least 30 min. At 42°C. cDNA probes were labeled with 32P-CTP by random priming (Statagene, La Jolla, CA), briefly denatured with 0.25M NaOH and added to the prehybridization solution. After a further incubation for at least 24h at 42°C, the blots were washed in high stringency conditions and exposed to X-ray film.

Very high expression of TR6 RNA was detected in aortic endothelial cells. High expression was also detected in monocytes. Low expression was detected in bone marrow and

CD4+ activated PBLs. Very low, but detectable levels of TR6 RNA was expressed in CD19+ PBLs, CD8+ PBLs (both activated and unstimulated), and unstimulated CD4+ PBLs.

In hematopoietic cell lines, low levels of TR6 RNA was expressed in HL60 (promyelocyte), KG1a (promyeloblast) and KG1 (myeloblast) cell lines. Very low but detectable levels of TR6 RNA was expressed in U937 (monoblast) and THP-1 (monocyte) cell lines.

The major RNA form is 3.8 kb in size.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: DEEN, KEITH C
YOUNG, PETER R

(ii) TITLE OF THE INVENTION: TUMOR NECROSIS FACTOR RELATED RECEPTOR, TR6

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: RATNER & PRESTIA
(B) STREET: P.O. BOX 980
(C) CITY: VALLEY FORGE
(D) STATE: PA
(E) COUNTRY: USA
(F) ZIP: 19482

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: TO BE ASSIGNED
(B) FILING DATE: 09-MAY-1997
(C) CLASSIFICATION: Unknown

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/041,230
(B) FILING DATE: 14-MAR-1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: PRESTIA, PAUL F.
(B) REGISTRATION NUMBER: 23,031
(C) REFERENCE/DOCKET NUMBER: GH-50008

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 610-407-0700
(B) TELEFAX: 610-407-0701
(C) TELEX: 846169

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3,881 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CTTTGCGCCC ACAAAATACA CCGACGATGC CCGATCTACT TTAAGGGCTG AAACCCACGG 60
GCCTGAGAGA CTATAAGAGC GTTCCCTACC GCCATGGAAC AACGGGGACA GAACGCCCCG 120
GCCGCTTCGG GGGCCCGGAA AAGGCACGGC CCAGGACCCA GGGAGGCGCG GGGAGCCAGG 180
CCTGGGCCCC GGGTCCCCAA GACCCTTGTG CTCGTTGTCG CCGCGGTCCT GCTGTTGGTC 240
TCAGCTGAGT CTGCTCTGAT CACCCAACAA GACCTAGCTC CCCAGCAGAG AGCGGCCCCA 300
CAACAAAAGA GGTCCAGCCC CTCAGAGGGA TTGTGTCCAC CTGGACACCA TATCTCAGAA 360
GACGGTAGAG ATTGCATCTC CTGCAAATAT GGACAGGACT ATAGCACTCA ATGGAATGAC 420
CTCCTTTTCT GCTTGCGCTG CACCAGGTGT GATTCAGGTG AAGTGGAGCT AAGTCCCTGC 480
ACCACGACCA GAAACACAGT GTGTCAGTGC GAAGAAGGCA CCTTCCGGGA AGAAGATTCT 540
CCTGAGATGT GCCGGAAGTG CCGCACAGGG TGTCCCAGAG GGATGGTCAA GGTCGGTGAT 600
TGTACACCCT GGAGTGACAT CGAATGTGTC CACAAAGAAT CAGGCATCAT CATAGGAGTC 660
ACAGTTGCAG CCGTAGTCTT GATTGTGGCT GTGTTTGTTT GCAAGTCTTT ACTGTGGAAG 720
AAAGTCCTTC CTTACCTGAA AGGCATCTGC TCAGGTGGTG GTGGGGACCC TGAGCGTGTG 780

GACAGAAGCT CACAACGACC TGGGGCTGAG GACAATGTCC TCAATGAGAT CGTGAGTATC 840
TTGCAGCCCA CCCAGGTCCC TGAGCAGGAA ATGGAAGTCC AGGAGCCAGC AGAGCCAACA 900
GGTGTCAACA TGTTGTCCCC CGGGGAGTCA GAGCATCTGC TGGAACCGGC AGAAGCTGAA 960
AGGTCTCAGA GGAGGAGGCT GCTGGTTCCA GCAAATGAAG GTGATCCCAC TGAGACTCTG 1020
AGACAGTGCT TCGATGACTT TGCAGACTTG GTGCCCTTTG ACTCCTGGGA GCCGCTCATG 1080
AGGAAGTTGG GCCTCATGGA CAATGAGATA AAGGTGGCTA AAGCTGAGGC AGCGGGCCAC 1140
AGGGACACCT TGTACACGAT GCTGATAAAG TGGGTCAACA AAACCGGGCG AGATGCCTCT 1200
GTCCACACCC TGCTGGATGC CTTGGAGACG CTGGGAGAGA GACTTGCCAA GCAGAAGATT 1260
GAGGACCACT TGTTGAGCTC TGGAAAGTTC ATGTATCTAG AAGGTAATGC AGACTCTGCC 1320
ATGTCCTAAG TGTGATTCTC TTCAGGAAGT CAGACCTTCC CTGGTTTACC TTTTTTCTGG 1380
AAAAAGCCCA ACTGGACTCC AGTCAGTAGG AAAGTGCCAC AATTGTCACA TGACCGGTAC 1440
TGGAAGAAAC TCTCCCATCC AACATCACCC AGTGGATGGA ACATCCTGTA ACTTTTCACT 1500
GCACTTGGCA TTATTTTTAT AAGCTGAATG TGATAATAAG GACACTATGG AAATGTCTGG 1560
ATCATTCCGT TTGTGCGTAC TTTGAGATTT GGTTTGGGAT GTCATTGTTT TCACAGCACT 1620
TTTTTATCCT AATGTAAATG CTTTATTTAT TTATTTGGGC TACATTGTAA GATCCATCTA 1680
CACAGTCGTT GTCCGACTTC ACTTGATACT ATATGATATG AACCTTTTTT GGGTGGGGGG 1740
TGCGGGGCAG TTCACTCTGT CTCCCAGGCT GGAGTGCAAT GGTGCAATCT TGGCTCACTA 1800
TAGCCTTGAC CTCTCAGGCT CAAGCGATTC TCCCACCTCA GCCATCCAAA TAGCTGGGAC 1860
CACAGGTGTG CACCACCACG CCCGGCTAAT TTTTTGTATT TTGTCTAGAT ATAGGGGCTC 1920
TCTATGTTGC TCAGGGTGGT CTCGAATTCC TGGACTCAAG CAGTCTGCCC ACCTCAGACT 1980
CCCAAAGCGG TGGAATTAGA GGCGTGAGCC CCCATGCTTG GCCTTACCTT TCTACTTTTA 2040
TAATTCTGTA TGTTATTATT TTATGAACAT GAAGAAACTT TAGTAAATGT ACTTGTTTAC 2100
ATAGTTATGT GAATAGATTA GATAAACATA AAAGGAGGAG ACATACAATG GGGGAAGAAG 2160
AAGAAGTCCC CTGTAAGATG TCACTGTCTG GGTTCCAGCC CTCCCTCAGA TGTACTTTGG 2220
CTTCAATGAT TGGCAACTTC TACAGGGGCC AGTCTTTTGA ACTGGACAAC CTTACAAGTA 2280
TATGAGTATT ATTTATAGGT AGTTGTTTAC ATATGAGTCG GGACCAAAGA GAACTGGATC 2340
CAGGTGAAGT CCTGTGTGTG GCTGGTCCCT ACCTGGGCAG TCTCATTTGC ACCCATAGCC 2400
CCCATCTATG GACAGGCTGG GACAGAGGCA GATGGGTTAG ATCACACATA ACAATAGGGT 2460
CTATGTCATA TCCCAAGTGA ACTTGAGCCC TGTTTGGGCT CAGGAGATAG AAGACAAAAT 2520
CTGTCTCCCC ACGTCTGCCA TGGCATCAAG GGGGAAGAGT AGATGGTGCT TGAGAATGGT 2580
GTGAAATGGT TGCCATCTCA GGAGTAGATG GCCCGGCTCA CTTCTGGTTA TCTGTCACCC 2640
TGAGCCCATG AGCTGCCTTT TAGGGTACAG ATTGCCTACT TGAGGACCTT GGCCGCTCTG 2700
TAAGCATCTG ACTCATCTCA GAAATGTCAA TTCTTAAACA CTGTGGCAAC AGGACCTAGA 2760
ATGGCTGACG CATTAAGGTT TTCTTCTTGT GTCCTGTTCT ATTATTGTTT TAAGACCTCA 2820
GTAACCATTT CAGCCTCTTT CCAGCAAACC CTTCTCCATA GTATTTCAGT CATGGAAGGA 2880
TCATTTATGC AGGTAGTCAT TCCAGGAGTT TTTGGTCTTT TCTGTCTCAA GGCATTGTGT 2940

GTTTTGTTCC GGGACTGGTT TGGGTGGGAC AAAGTTAGAA TTGCCTGAAG ATCACACATT 3000
CAGACTGTTG TGTCTGTGGA GTTTTAGGAG TGGGGGGTGA CCTTTCTGGT CTTTGCACTT 3060
CCATCCTCTC CCACTTCCAT CTGGCATCCC CACGCGTTGT CCCCTGCACT TCTGGAAGGC 3120
ACAGGGTGCT GCTGCTTCCT GGTCTTTGCC TTTGCTGGGC CTTCTGTGCA GGACGCTCAG 3180
CCTCAGGGCT CAGAAGGTGC CAGTCCGGTC CCAGGTCCCT TGTCCCTTCC ACAGAGGCCT 3240
TCCTAGAAGA TGCATCTAGA GTGTCAGCCT TATCAGTGTT TAAGATTTTT CTTTTATTTT 3300
TAATTTTTTT GAGACAGAAT CTCACTCTCT CGCCCAGGCT GGAGTGCAAC GGTACGATCT 3360
TGGCTCAGTG CAACCTCCGC CTCCTGGGTT CAAGCGATTC TCGTGCCTCA GCCTCCGGAG 3420
TAGCTGGGAT TGCAGGCACC CGCCACCACG CCTGGCTAAT TTTTGTATTT TTAGTAGAGA 3480
CGGGGTTTCA CCATGTTGGT CAGGCTGGTC TCGAACTCCT GACCTCAGGT GATCCACNTT 3540
GGCCTCCGAA AGTGCTGGGA TATACAAGGC GTGAGCCACC AGCCAGGCCA AGATATTNNT 3600
NTAAAGNGAG CTTCCGGANG ACATGAAATA ANGGGGGGTT TTGTTGTTTA GTAACATTNG 3660
GCTTTGATAT ATCCCCAGGC CAAATNGCAN GNGACACAGG ACAGCCATAG TATAGTGTGT 3720
CACTCGTGGT TGGTGTCCTT TCATGGTTCT GCCCTGTCAA AGGTCCCTAT TTGAAATGTG 3780
TTATAATACA AACAAGGAAG CACATTGTGT ACAAAATACT TATGTATTTA TGAATCCATG 3840
ACCAAATTAA ATATGAAACC TTATATAAAA AAAAAAAAAA A 3881

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 411 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Glu Gln Arg Gly Gln Asn Ala Pro Ala Ala Ser Gly Ala Arg Lys
1 5 10 15
Arg His Gly Pro Gly Pro Arg Glu Ala Arg Gly Ala Arg Pro Gly Pro
20 25 30
Arg Val Pro Lys Thr Leu Val Leu Val Val Ala Ala Val Leu Leu Leu
35 40 45
Val Ser Ala Glu Ser Ala Leu Ile Thr Gln Gln Asp Leu Ala Pro Gln
50 55 60

Gln Arg Ala Ala Pro Gln Gln Lys Arg Ser Ser Pro Ser Glu Gly Leu
65 70 75 80
Cys Pro Pro Gly His His Ile Ser Glu Asp Gly Arg Asp Cys Ile Ser
85 90 95
Cys Lys Tyr Gly Gln Asp Tyr Ser Thr Gln Trp Asn Asp Leu Leu Phe
100 105 110
Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val Glu Leu Ser Pro
115 120 125
Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu Glu Gly Thr Phe
130 135 140
Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys Arg Thr Gly Cys
145 150 155 160
Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile
165 170 175
Glu Cys Val His Lys Glu Ser Gly Ile Ile Ile Gly Val Thr Val Ala
180 185 190
Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp
195 200 205
Lys Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly
210 215 220
Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp
225 230 235 240
Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val Pro
245 250 255
Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly Val Asn
260 265 270
Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala Glu Ala
275 280 285
Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala Asn Glu Gly Asp
290 295 300
Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe Ala Asp Leu Val
305 310 315 320
Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu Gly Leu Met Asp
325 330 335
Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly His Arg Asp Thr
340 345 350
Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr Gly Arg Asp Ala
355 360 365
Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu Gly Glu Arg Leu
370 375 380
Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser Gly Lys Phe Met
385 390 395 400

Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser End
405 410 411

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1062 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGACCTCCT TTTCTGCTTG CGCTGCACCA GGTGTGATTC AGGTGAAGTG GAGCTAAGTC 60
CCTGCACCAC GACCAGAAAC ACAGTGTGTC AGTGCGAAGA AGGCACCTTC CGGGAAGAAG 120
ATTCTCCTGA GATGTGCCGG AAGTGCCGCA CAGGGTGTCC CAGAGGGATG GTCAAGGTCG 180
GTGATTGTAC ACCCTGGAGT GACATCGAAT GTGTCCACAA AGAATCAGGC ATCATCATAG 240
GAGTCACAGT TGCAGCCGTA GTCTTGATTG TGGCTGTGTT TGTTTGCAAG TCTTTACTGT 300
GGAAGAAAGT CCTTCCTTAC CTGAAAGGCA TCTGCTCAGG TGGTGGTGGG GACCCTGAGC 360
GTGTGGACAG AAGCTCACAA CGACCTGGGG CTGAGGACAA TGTCCTCAAT GAGATCGTGA 420
GTATCTTGCA GCCCACCCAG GTCCCTGAGC AGGAAATGGA AGTCCAGGAG CCAGCAGAGC 480
CAACAGGTGT CAACATGTTG TCCCCCGGGG AGTCAGAGCA TCTGCTGGAA CCGGCAGAAG 540
CTGAAAGGTC TCAGAGGAGG AGGCTGCTGG TTCCAGCAAA TGAAGGTGAT CCCACTGAGA 600
CTCTGAGACA GTGCTTCGAT GACTTTGCAG ACTTGGTGCC CTTTGACTCC TGGGAGCCGC 660
TCATGAGGAA GTTGGGCCTC ATGGACAATG AGATAAAGGT GGCTAAAGCT GAGGCAGCGG 720
GCCACAGGGA CACCTTGTAC ACGATGCTGA TAAAGTGGGT CAACAAAACC GGGCGAGATG 780
CCTCTGTCCA CACCCTGCTG GATGCCTTGG AGACGCTGGG AGAGAGACTT GCCAAGCAGA 840
AGATTGAGGA CCACTTGTTG AGCTCTGGAA AGTTCATGTA TCTAGAAGGT AATGCAGACT 900
CTGCCATGTC CTAAGTGTGA TTCTCTTCAG GAAGTCAGAC CTTCCCTGGT TTACCTTTTT 960
TCTGGAAAAA GCCCAACTGG ACTCCAGTCA GTAGGAAAGT GCCACAATTG TCACATGACC 1020
GGTACTGGAA GAAACTCTCC CATCCAACAT CACCCAGTGG AT 1062

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 303 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Asp Leu Leu Phe Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val
 1               5                  10                  15
Glu Leu Ser Pro Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu
            20                  25                  30
Glu Gly Thr Phe Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys
        35                  40                  45
Arg Thr Gly Cys Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro
    50                  55                  60
Trp Ser Asp Ile Glu Cys Val His Lys Glu Ser Gly Ile Ile Ile Gly
65                  70                  75                  80
Val Thr Val Ala Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys
                85                  90                  95
Ser Leu Leu Trp Lys Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser
            100                 105                 110
Gly Gly Gly Gly Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro
        115                 120                 125
Gly Ala Glu Asp Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro
    130                 135                 140
Thr Gln Val Pro Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro
145                 150                 155                 160
Thr Gly Val Asn Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu
                165                 170                 175
Pro Ala Glu Ala Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala
            180                 185                 190
Asn Glu Gly Asp Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe
        195                 200                 205
Ala Asp Leu Val Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu
    210                 215                 220
Gly Leu Met Asp Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly
225                 230                 235                 240
His Arg Asp Thr Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr
                245                 250                 255
Gly Arg Asp Ala Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu
            260                 265                 270
Gly Glu Arg Leu Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser
        275                 280                 285
```

GH-50008

Gly Lys Phe Met Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser
290 295 300

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the TR6 polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence.

2. The polynucleotide of claim 1 which is DNA or RNA.

3. The polynucleotide of claim 1 wherein said nucleotide sequence is at least 80% identical to that contained in SEQ ID NO:1.

4. The polynucleotide of claim 3 wherein said nucleotide sequence comprises the TR6 polypeptide encoding sequence contained in SEQ ID NO:1.

5. The polynucleotide of claim 3 which is polynucleotide of SEQ ID NO: 1.

6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing a TR6 polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide of SEQ ID NO:2 when said expression system is present in a compatible host cell.

7. A host cell comprising the expression system of claim 6.

8. A process for producing a TR6 polypeptide comprising culturing a host of claim 7 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture.

9. A process for producing a cell which produces a TR6 polypeptide thereof comprising transforming or transfecting a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces a TR6 polypeptide.

10. A TR6 polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO:2 over its entire length.

11. The polypeptide of claim 10 which comprises the amino acid sequence of SEQ ID NO:2.

12. An antibody immunospecific for the TR6 polypeptide of claim 10.

13. A method for the treatment of a subject in need of enhanced activity or expression of TR6 polypeptide of claim 10 comprising:

(a) administering to the subject a therapeutically effective amount of an agonist to said receptor, and/or

(b) providing to the subject an isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the TR6 polypeptide of SEQ ID NO:2 over its entire length, or a nucleotide sequence complementary to said nucleotide sequence in a form so as to effect production of said polypeptide activity *in vivo*.

14. A method for the treatment of a subject having need to inhibit activity or expression of TR6 polypeptide of claim 10 comprising:

(a) administering to the subject a therapeutically effective amount of an antagonist to said receptor, and/or

(b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said receptor, and/or

(c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said receptor for its ligand.

15. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of TR6 polypeptide of claim 10 in a subject comprising:

(a) determining the presence or absence of a mutation in the nucleotide sequence encoding said TR6 polypeptide in the genome of said subject; and/or

(b) analyzing for the presence or amount of the TR6 polypeptide expression in a sample derived from said subject.

16. A method for identifying agonists to TR6 polypeptide of claim 10 comprising:

(a) contacting a cell which produces a TR6 polypeptide with a candidate compound; and

(b) determining whether the candidate compound effects a signal generated by activation of the TR6 polypeptide.

17. An agonist identified by the method of claim 16.

18. The method for identifying antagonists to TR6 polypeptide of claim 10 comprising:

(a) contacting said a cell which produces a TR6 polypeptide with an agonist; and

(b) determining whether the signal generated by said agonist is diminished in the presence of a candidate compound.

19. An antagonist identified by the method of claim 18.

ABSTRACT OF THE DISCLOSURE

TR6 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing TR6 polypeptides and polynucleotides in the design of protocols for the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease., among others and diagnostic assays for such conditions.

GH-50008

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: DEEN, KEITH C
YOUNG, PETER R

(ii) TITLE OF THE INVENTION: TUMOR NECROSIS FACTOR RELATED RECEPTOR, TR6

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: RATNER & PRESTIA
(B) STREET: P.O. BOX 980
(C) CITY: VALLEY FORGE
(D) STATE: PA
(E) COUNTRY: USA
(F) ZIP: 19482

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: TO BE ASSIGNED
(B) FILING DATE: 09-MAY-1997
(C) CLASSIFICATION: Unknown

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/041,230
(B) FILING DATE: 14-MAR-1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: PRESTIA, PAUL F.
(B) REGISTRATION NUMBER: 23,031
(C) REFERENCE/DOCKET NUMBER: GH-50008

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 610-407-0700
(B) TELEFAX: 610-407-0701
(C) TELEX: 846169

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3,881 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CTTTGCGCCC ACAAAATACA CCGACGATGC CCGATCTACT TTAAGGGCTG AAACCCACGG 60
GCCTGAGAGA CTATAAGAGC GTTCCCTACC GCCATGGAAC AACGGGGACA GAACGCCCCG 120
GCCGCTTCGG GGGCCCGGAA AAGGCACGGC CCAGGACCCA GGGAGGCGCG GGGAGCCAGG 180
CCTGGGCCCC GGGTCCCCAA GACCCTTGTG CTCGTTGTCG CCGCGGTCCT GCTGTTGGTC 240
TCAGCTGAGT CTGCTCTGAT CACCCAACAA GACCTAGCTC CCCAGCAGAG AGCGGCCCCA 300
CAACAAAAGA GGTCCAGCCC CTCAGAGGGA TTGTGTCCAC CTGGACACCA TATCTCAGAA 360
GACGGTAGAG ATTGCATCTC CTGCAAATAT GGACAGGACT ATAGCACTCA ATGGAATGAC 420
CTCCTTTTCT GCTTGCGCTG CACCAGGTGT GATTCAGGTG AAGTGGAGCT AAGTCCCTGC 480
ACCACGACCA GAAACACAGT GTGTCAGTGC GAAGAAGGCA CCTTCCGGGA AGAAGATTCT 540
CCTGAGATGT GCCGGAAGTG CCGCACAGGG TGTCCCAGAG GGATGGTCAA GGTCGGTGAT 600
TGTACACCCT GGAGTGACAT CGAATGTGTC CACAAAGAAT CAGGCATCAT CATAGGAGTC 660
ACAGTTGCAG CCGTAGTCTT GATTGTGGCT GTGTTTGTTT GCAAGTCTTT ACTGTGGAAG 720

```
AAAGTCCTTC CTTACCTGAA AGGCATCTGC TCAGGTGGTG GTGGGGACCC TGAGCGTGTG   780
GACAGAAGCT CACAACGACC TGGGGCTGAG GACAATGTCC TCAATGAGAT CGTGAGTATC   840
TTGCAGCCCA CCCAGGTCCC TGAGCAGGAA ATGGAAGTCC AGGAGCCAGC AGAGCCAACA   900
GGTGTCAACA TGTTGTCCCC CGGGGAGTCA GAGCATCTGC TGGAACCGGC AGAAGCTGAA   960
AGGTCTCAGA GGAGGAGGCT GCTGGTTCCA GCAAATGAAG GTGATCCCAC TGAGACTCTG  1020
AGACAGTGCT TCGATGACTT TGCAGACTTG GTGCCCTTTG ACTCCTGGGA GCCGCTCATG  1080
AGGAAGTTGG GCCTCATGGA CAATGAGATA AAGGTGGCTA AAGCTGAGGC AGCGGGCCAC  1140
AGGGACACCT TGTACACGAT GCTGATAAAG TGGGTCAACA AAACGGGCGG AGATGCCTCT  1200
GTCCACACCC TGCTGGATGC CTTGGAGACG CTGGGAGAGA GACTTGCCAA GCAGAAGATT  1260
GAGGACCACT TGTTGAGCTC TGGAAAGTTC ATGTATCTAG AAGGTAATGC AGACTCTGCC  1320
ATGTCCTAAG TGTGATTCTC TTCAGGAAGT CAGACCTTCC CTGGTTTACC TTTTTTCTGG  1380
AAAAAGCCCA ACTGGACTCC AGTCAGTAGG AAAGTGCCAC AATTGTCACA TGACCGGTAC  1440
TGGAAGAAAC TCTCCCATCC AACATCACCC AGTGGATGGA ACATCCTGTA ACTTTTCACT  1500
GCACTTGGCA TTATTTTTAT AAGCTGAATG TGATAATAAG GACACTATGG AAATGTCTGG  1560
ATCATTCCGT TTGTGCGTAC TTTGAGATTT GGTTTGGGAT GTCATTGTTT TCACAGCACT  1620
TTTTTATCCT AATGTAAATG CTTTATTTAT TTATTTGGGC TACATTGTAA GATCCATCTA  1680
CACAGTCGTT GTCCGACTTC ACTTGATACT ATATGATATG AACCTTTTTT GGGTGGGGGG  1740
TGCGGGGCAG TTCACTCTGT CTCCCAGGCT GGAGTGCAAT GGTGCAATCT TGGCTCACTA  1800
TAGCCTTGAC CTCTCAGGCT CAAGCGATTC TCCCACCTCA GCCATCCAAA TAGCTGGGAC  1860
CACAGGTGTG CACCACCACG CCCGGCTAAT TTTTTGTATT TTGTCTAGAT ATAGGGGCTC  1920
TCTATGTTGC TCAGGGTGGT CTCGAATTCC TGGACTCAAG CAGTCTGCCC ACCTCAGACT  1980
CCCAAAGCGG TGGAATTAGA GGCGTGAGCC CCCATGCTTG GCCTTACCTT TCTACTTTTA  2040
TAATTCTGTA TGTTATTATT TTATGAACAT GAAGAAACTT TAGTAAATGT ACTTGTTTAC  2100
ATAGTTATGT GAATAGATTA GATAAACATA AAAGGAGGAG ACATACAATG GGGGAAGAAG  2160
AAGAAGTCCC CTGTAAGATG TCACTGTCTG GGTTCCAGCC CTCCCTCAGA TGTACTTTGG  2220
CTTCAATGAT TGGCAACTTC TACAGGGGCC AGTCTTTTGA ACTGGACAAC CTTACAAGTA  2280
TATGAGTATT ATTTATAGGT AGTTGTTTAC ATATGAGTCG GGACCAAAGA GAACTGGATC  2340
CACGTGAAGT CCTGTGTGTG GCTGGTCCCT ACCTGGGCAG TCTCATTTGC ACCCATAGCC  2400
CCCATCTATG GACAGGCTGG GACAGAGGCA GATGGGTTAG ATCACACATA ACAATAGGGT  2460
CTATGTCATA TCCCAAGTGA ACTTGAGCCC TGTTTGGGCT CAGGAGATAG AAGACAAAAT  2520
CTGTCTCCCC ACGTCTGCCA TGGCATCAAG GGGGAAGAGT AGATGGTGCT TGAGAATGGT  2580
GTGAAATGGT TGCCATCTCA GGAGTAGATG GCCCGGCTCA CTTCTGGTTA TCTGTCACCC  2640
TGAGCCCATG AGCTGCCTTT TAGGGTACAG ATTGCCTACT TGAGGACCTT GGCCGCTCTG  2700
TAAGCATCTG ACTCATCTCA GAAATGTCAA TTCTTAAACA CTGTGGCAAC AGGACCTAGA  2760
ATGGCTGACG CATTAAGGTT TTCTTCTTGT GTCCTGTTCT ATTATTGTTT TAAGACCTCA  2820
```

GTAACCATTT CAGCCTCTTT CCAGCAAACC CTTCTCCATA GTATTTCAGT CATGGAAGGA 2880
TCATTTATGC AGGTAGTCAT TCCAGGAGTT TTTGGTCTTT TCTGTCTCAA GGCATTGTGT 2940
GTTTTGTTCC GGGACTGGTT TGGGTGGGAC AAAGTTAGAA TTGCCTGAAG ATCACACATT 3000
CAGACTGTTG TGTCTGTGGA GTTTTAGGAG TGGGGGGTGA CCTTTCTGGT CTTTGCACTT 3060
CCATCCTCTC CCACTTCCAT CTGGCATCCC CACGCGTTGT CCCCTGCACT TCTGGAAGGC 3120
ACAGGGTGCT GCTGCTTCCT GGTCTTTGCC TTTGCTGGGC CTTCTGTGCA GGACGCTCAG 3180
CCTCAGGGCT CAGAAGGTGC CAGTCCGGTC CCAGGTCCCT TGTCCCTTCC ACAGAGGCCT 3240
TCCTAGAAGA TGCATCTAGA GTGTCAGCCT TATCAGTGTT TAAGATTTTT CTTTTATTTT 3300
TAATTTTTTT GAGACAGAAT CTCACTCTCT CGCCCAGGCT GGAGTGCAAC GGTACGATCT 3360
TGGCTCAGTG CAACCTCCGC CTCCTGGGTT CAAGCGATTC TCGTGCCTCA GCCTCCGGAG 3420
TAGCTGGGAT TGCAGGCACC CGCCACCACG CCTGGCTAAT TTTTGTATTT TTAGTAGAGA 3480
CGGGGTTTCA CCATGTTGGT CAGGCTGGTC TCGAACTCCT GACCTCAGGT GATCCACNTT 3540
GGCCTCCGAA AGTGCTGGGA TATACAAGGC GTGAGCCACC AGCCAGGCCA AGATATTNTT 3600
NTAAAGNNAG CTTCCGGANG ACATGAAATA ANGGGGGGTT TTGTTGTTTA GTAACATTNG 3660
GCTTTGATAT ATCCCCAGGC CAAATNGCAN GNGACACAGG ACAGCCATAG TATAGTGTGT 3720
CACTCGTGGT TGGTGTCCTT TCATGGTTCT GCCCTGTCAA AGGTCCCTAT TTGAAATGTG 3780
TTATAATACA AACAAGGAAG CACATTGTGT ACAAAATACT TATGTATTTA TGAATCCATG 3840
ACCAAATTAA ATATGAAACC TTATATAAAA AAAAAAAAAA A 3881

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 411 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Glu Gln Arg Gly Gln Asn Ala Pro Ala Ala Ser Gly Ala Arg Lys
1 5 10 15

Arg His Gly Pro Gly Pro Arg Glu Ala Arg Gly Ala Arg Pro Gly Pro
20 25 30

```
Arg Val Pro Lys Thr Leu Val Leu Val Val Ala Ala Val Leu Leu Leu
            35                  40                  45
Val Ser Ala Glu Ser Ala Leu Ile Thr Gln Gln Asp Leu Ala Pro Gln
        50                  55                  60
Gln Arg Ala Ala Pro Gln Gln Lys Arg Ser Ser Pro Ser Glu Gly Leu
 65                  70                  75                  80
Cys Pro Pro Gly His His Ile Ser Glu Asp Gly Arg Asp Cys Ile Ser
                85                  90                  95
Cys Lys Tyr Gly Gln Asp Tyr Ser Thr Gln Trp Asn Asp Leu Leu Phe
            100                 105                 110
Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val Glu Leu Ser Pro
        115                 120                 125
Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu Glu Gly Thr Phe
    130                 135                 140
Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys Arg Thr Gly Cys
145                 150                 155                 160
Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile
                165                 170                 175
Glu Cys Val His Lys Glu Ser Gly Ile Ile Ile Gly Val Thr Val Ala
            180                 185                 190
Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp
        195                 200                 205
Lys Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly
    210                 215                 220
Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp
225                 230                 235                 240
Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val Pro
                245                 250                 255
Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly Val Asn
            260                 265                 270
Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala Glu Ala
        275                 280                 285
Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala Asn Glu Gly Asp
    290                 295                 300
Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe Ala Asp Leu Val
305                 310                 315                 320
Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu Gly Leu Met Asp
                325                 330                 335
Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly His Arg Asp Thr
            340                 345                 350
Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr Gly Arg Asp Ala
        355                 360                 365
Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu Gly Glu Arg Leu
    370                 375                 380
Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser Gly Lys Phe Met
385                 390                 395                 400
Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser End
                405                 410 411
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1062 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATGACCTCCT TTTCTGCTTG CGCTGCACCA GGTGTGATTC AGGTGAAGTG GAGCTAAGTC     60
CCTGCACCAC GACCAGAAAC ACAGTGTGTC AGTGCGAAGA AGGCACCTTC CGGGAAGAAG    120
ATTCTCCTGA GATGTGCCGG AAGTGCCGCA CAGGGTGTCC CAGAGGGATG GTCAAGGTCG    180
GTGATTGTAC ACCCTGGAGT GACATCGAAT GTGTCCACAA AGAATCAGGC ATCATCATAG    240
GAGTCACAGT TGCAGCCGTA GTCTTGATTG TGGCTGTGTT TGTTTGCAAG TCTTTACTGT    300
GGAAGAAAGT CCTTCCTTAC CTGAAAGGCA TCTGCTCAGG TGGTGGTGGG GACCCTGAGC    360
GTGTGGACAG AAGCTCACAA CGACCTGGGG CTGAGGACAA TGTCCTCAAT GAGATCGTGA    420
GTATCTTGCA GCCCACCCAG GTCCCTGAGC AGGAAATGGA AGTCCAGGAG CCAGCAGAGC    480
CAACAGGTGT CAACATGTTG TCCCCCGGGG AGTCAGAGCA TCTGCTGGAA CCGGCAGAAG    540
CTGAAAGGTC TCAGAGGAGG AGGCTGCTGG TTCCAGCAAA TGAAGGTGAT CCCACTGAGA    600
CTCTGAGACA GTGCTTCGAT GACTTTGCAG ACTTGGTGCC CTTTGACTCC TGGGAGCCGC    660
TCATGAGGAA GTTGGGCCTC ATGGACAATG AGATAAAGGT GGCTAAAGCT GAGGCAGCGG    720
GCCACAGGGA CACCTTGTAC ACGATGCTGA TAAAGTGGGT CAACAAAACC GGGCGAGATG    780
CCTCTGTCCA CACCCTGCTG GATGCCTTGG AGACGCTGGG AGAGAGACTT GCCAAGCAGA    840
AGATTGAGGA CCACTTGTTG AGCTCTGGAA AGTTCATGTA TCTAGAAGGT AATGCAGACT    900
CTGCCATGTC CTAAGTGTGA TTCTCTTCAG GAAGTCAGAC CTTCCCTGGT TTACCTTTTT    960
TCTGGAAAAA GCCCAACTGG ACTCCAGTCA GTAGGAAAGT GCCACAATTG TCACATGACC   1020
GGTACTGGAA GAAACTCTCC CATCCAACAT CACCCAGTGG AT                       1062
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 303 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Asp Leu Leu Phe Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val
 1               5                  10                  15
Glu Leu Ser Pro Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu
            20                  25                  30
Glu Gly Thr Phe Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys
```

```
          35                  40                  45
Arg Thr Gly Cys Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro
    50                  55                  60
Trp Ser Asp Ile Glu Cys Val His Lys Glu Ser Gly Ile Ile Ile Gly
65                  70                  75                  80
Val Thr Val Ala Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys
                85                  90                  95
Ser Leu Leu Trp Lys Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser
            100                 105                 110
Gly Gly Gly Gly Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro
        115                 120                 125
Gly Ala Glu Asp Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro
    130                 135                 140
Thr Gln Val Pro Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro
145                 150                 155                 160
Thr Gly Val Asn Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu
                165                 170                 175
Pro Ala Glu Ala Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala
            180                 185                 190
Asn Glu Gly Asp Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe
        195                 200                 205
Ala Asp Leu Val Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu
    210                 215                 220
Gly Leu Met Asp Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly
225                 230                 235                 240
His Arg Asp Thr Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr
                245                 250                 255
Gly Arg Asp Ala Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu
            260                 265                 270
Gly Glu Arg Leu Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser
        275                 280                 285
Gly Lys Phe Met Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser
    290                 295                 300
```

U.S. PROVISIONAL PATENT APPLICATION NO. 60/041,230

[redacted]

A

Tumor Necrosis Related Receptor, TR6

60/041230

FIELD OF INVENTION

This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to Tumor necrosis factor receptor (TNF-R) family, hereinafter referred to as TR6. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

BACKGROUND OF THE INVENTION

Many biological actions, for instance, response to certain stimuli and natural biological processes, are controlled by factors, such as cytokines. Many cytokines act through receptors by engaging the receptor and producing an intracellular response.

For example, tumor necrosis factors (TNF) alpha and beta are cytokines which act through TNF receptors to regulate numerous biological processes, including protection against infection and induction of shock and inflammatory disease. The TNF molecules belong to the "TNF-ligand" superfamily, and act together with their receptors or counter-ligands, the "TNF-receptor" superfamily. So far, nine members of the TNF ligand superfamily have been identified and ten members of the TNF-receptor superfamily have been characterized.

Among the ligands there are included TNF-a, lymphotoxin-a (LT-a, also known as TNF-b), LT-b (found in complex heterotrimer LT-a2-b), FasL, CD40L, CD27L, CD30L, 4-1BBL, OX40L and nerve growth factor (NGF)). The superfamily of TNF receptors includes the p55TNF receptor, p75TNF receptor, TNF receptor-related protein, FAS antigen or APO-1, CD40, CD27, CD30, 4-1BB, OX40, low affinity p75 and NGF-receptor (Meager, A., Biologicals, 22:291-295 (1994)).

Many members of the TNF-ligand superfamily are expressed by activated T-cells, implying that they are necessary for T-cell interactions with other cell types which underlie cell ontogeny and functions. (Meager, A., supra).

Considerable insight into the essential functions of several members of the TNF receptor family has been gained from the identification and creation of mutants that abolish the expression of these proteins. For example, naturally occurring mutations in the FAS antigen and its ligand cause lymphoproliferative disease (Watanabe-Fukunaga, R., et al., Nature 356:314 (1992)), perhaps reflecting a failure of programmed cell death. Mutations of the CD40 ligand cause an X-linked immunodeficiency state characterized by high levels of immunoglubulin M and low levels of immunoglobulin G in plasma, indicating faulty T-cell-dependent B-cell activation (Allen, R.C. et al., Science 259:990 (1993)). Targeted mutations of the low affinity nerve growth factor receptor cause a disorder characterized by faulty sensory innovation of peripheral structures (Lee, K.F. et al, Cell 69:737 (1992)).

TNF and LT-a are capable of binding to two TNF receptors (the 55- and 75-kd TNF receptors). A large number of biological effects elicited by TNF and LT-a, acting through their receptors, include hemorrhagic necrosis of transplanted tumors, cytotoxicity, a role in endotoxic shock, inflammation, immunoregulation, proliferation and anti-viral responses, as well as protection against the deleterious effects of ionizing radiation. TNF and LT-a are involved in the pathogenesis of a wide range of diseases, including endotoxic shock, cerebral malaria, tumors, autoimmuine disease, AIDS and graft-host rejection (Beutler, B. and Von Huffel, C., Science 264:667-668 (1994)). Mutations in the p55 Receptor cause increased susceptibility to microbial infection.

Moreover, an about 80 amino acid domain near the C-terminus of TNFR1 (P55) and Fas was reported as the "death domain," which is responsible for transducing signals for programmed cell death (Tartaglia et al., Cell 74:845 (1993)).

The effects of TNF family ligands and TNF family receptors are varied and influence numerous functions, both normal and abnormal, in the biological processes of the mammalian system. There is a clear need, therefore, for identification and characterization of such receptors and ligands that influence biological activity, both normally and in disease states. In particular, there is a need to isolate and characterize novel members of the TNF receptor family.

This indicates that these Tumor necrosis factor receptors (TNF-R) have an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further members of Tumor necrosis factor receptor (TNF-R) family which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (eg inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (eg lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to TR6 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such TR6 polypeptides and polynucleotides. Such uses include the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (eg inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (eg lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with TR6 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate TR6 activity or levels.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleotide and deduced amino acid sequence of the partial sequence of human TR6. SEQ ID NOS: 1 and 2.

DESCRIPTION OF THE INVENTION

Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"TR6" refers generally to a polypeptide having the amino acid sequence set forth in SEQ ID NO:2 or an allelic variant thereof.

"TR6 activity or TR6 polypeptide activity" or "biological activity of the TR6 or TR6 polypeptide" refers to the metabolic or physiologic function of said TR6 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said TR6.

"TR6 polypeptides" refers to polypeptides with amino acid sequences sufficiently similar to TR6 sequences, preferably exhibiting at least one biological activity of the TR6.

"TR6 gene" refers to a polynucleotide having the nucleotide sequence set forth in SEQ ID NO:1 or allelic variants thereof and/or their complements.

"TR6 polynucleotides" refers to polynucleotides containing a nucleotide sequence which encodes a TR6 polypeptide or fragment thereof, or a nucleotide sequence which has at least 58% identity to a nucleotide sequence encoding the polypeptide of SEQ ID NO:2 or the corresponding fragment thereof, or a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example,

tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press,

New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge

Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., *et al.*, *Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. *et al.*, *J Molec Biol* (1990) 215:403).

Polypeptides of the Invention

The TR6 polypeptides of the present invention include the polypeptide of SEQ ID NO:2 (in particular the mature polypeptide) as well as TR6 polypeptides and which have at least 80% identity to the polypeptide of SEQ ID NO:2 or the relevant portion and more preferably at least 85% identity, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2.

The TR6 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

Biologically active fragments of the TR6 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned TR6 polypeptides. As with TR6 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of TR6 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of TR6 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet

and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Biologically active fragments are those that mediate TR6 activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Thus, the polypeptides of the invention include polypeptides having an amino acid sequence at least 80% identical to that of SEQ ID NO:2 or fragments thereof with at least 80% identity to the corresponding fragment of SEQ ID NO:2. Preferably, all of these polypeptides retain the biological activity of the TR6, including antigenic activity. Included in this group are variants of the defined sequence and fragments. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

The TR6 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

Another aspect of the invention relates to isolated polynucleotides which encode the TR6 polypeptides and polynucleotides closely related thereto.

TR6 of the invention is structurally related to other proteins of the Tumor necrosis factor receptor (TNF-R), as shown by the results of sequencing the cDNA encoding human TR6. The cDNA sequence contains an open reading frame encoding a protein of 307 amino acids with a deduced molecular weight of 33.9 kDa. TR6 of Figure 1 (SEQ ID NO:2) has about 25.0 % identity (using BESTFIT (from GCG suite of Programs)) in 307 amino acid residues with murine tumor necrosis factor receptor 2 (TNF-R2) (M. Lewis et al, Proc. Natl. Acad. Sci. USA. 88, 2830-2834, (1991); R.G. Goodwin et al, Mol. Cell. Biol. 11, 3020-3026, (1991)). Furthermore, TR6 (SEQ ID NO:2) is 25.6 % identical to human tumor necrosis factor receptor 1 (TNF-R1) (P. Fuchs et al., Genomics 13 (1), 219-224 (1992), over 307 amino

acids and 21.3% identical to human TNF-R2 over 307 amino acid residues (C.A. Smith et al., Science 248:1019-1023(1990)). TR6 contains a death domain (amino acids 220 to 277 in SEQ ID NO:2) which is 35.7% identical to the death domain of the human Death receptor 3 (DR3) (A.M. Chinnaiyan, et al, Science 274 (5289), 990-992 (1996)), 32.7% identical to the death domain of human TNFR-1, and 19.6% identical to the death domain of CD95 (Fas) (I. Cascino, J. Immunol. 154 (6), 2706-2713 (1995)). Based on homology with other members of the TNF receptor family, the predicted length of the full length TR6 is approximately 410 amino acids with a predicted molecular weight of 45 kDA.

One polynucleotide of the present invention encoding TR6 may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of human thymus stromal cells, monocytes, peripheral blood lymphocytes, and bone marrow cells using the expressed sequence tag (EST) analysis (Adams, M.D., *et al. Science* (1991) 252:1651-1656; Adams, M.D. *et al., Nature*, (1992) *355*:632-634; Adams, M.D., *et al., Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

Thus, the nucleotide sequence encoding TR6 polypeptides may be identical over its entire length to the coding sequence in Figure 1 (SEQ ID NO:1), or may be a degenerate form of this nucleotide sequence encoding the polypeptide of SEQ ID NO:2, or may be highly identical to a nucleotide sequence that encodes the polypeptide of SEQ ID NO:2. Preferably, the polynucleotides of the invention contain a nucleotide sequence that is highly identical, at least 80% identical, with a nucleotide sequence encoding a TR6 polypeptide, or at least 80% identical with the encoding nucleotide sequence set forth in Figure 1 (SEQ ID NO:1), or at least 80% identical to a nucleotide sequence encoding the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of TR6 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al., Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also

contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Among particularly preferred embodiments of the invention are polynucleotides encoding TR6 polypeptides having the amino acid sequence of set out in Figure 1 (SEQ ID NO:2) and variants thereof.

Further preferred embodiments are polynucleotides encoding TR6 variants that have the amino acid sequence of the TR6 polypeptide of Figure 1 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination.

Further preferred embodiments of the invention are polynucleotides that are at least 80% identical over their entire length to a polynucleotide encoding the TR6 polypeptide having the amino acid sequence set out in Figure 1 (SEQ ID NO:2), and polynucleotides which are complementary to such polynucleotides. In this regard, polynucleotides at least 80% identical over their entire length to the same are particularly preferred, and those with at least 90% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred.

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences.

Polynucleotides of the invention, which are sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding TR6 polypeptide and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the TR6 gene. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 70% identical, preferably 80% identical, more preferably 90% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

Vectors, Host Cells, Expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL* (*supra*).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be

incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If the TR6 polypeptide is to be expressed for use in screening assays, the polypeptide may be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If TR6 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

TR6 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention also relates to the use of TR6 polynucleotides for use as diagnostic reagents. Detection of a mutated form of TR6 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of TR6. Individuals carrying mutations in the TR6 gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled TR6 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al.*, *Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al.*, *Proc Natl Acad Sci USA*

(1985) 85: 4397-4401. In another embodiment, an array of oligonucleotides probes comprising TR6 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee et al., Science, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (eg inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (eg lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease.through detection of mutation in the TR6 gene by the methods described.

In addition, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (eg inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (eg lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease.can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of TR6 polypeptide or TR6 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an TR6 polypeptide, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on

line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

The 3' untranslated region of TR6 matches the 295 bp nucleotide sequence of a mapped EST (Genbank ID: D20151). This EST has been mapped by the Whitehead Institute to chromosome 8, 97.68 cR from the top of the Chromosome 8 linkage group.

The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the TR6 polypeptides. The term "immunospecific" means that the antibodies have substantiall greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the TR6 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against TR6 polypeptides may also be employed to treat chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (eg inflammatory bowel disease,

psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (eg lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease.among others.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with TR6 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (eg inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (eg lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease.among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering TR6 gene via a vector directing expression of TR6 polypeptide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a TR6 polypeptide wherein the composition comprises a TR6 polypeptide or TR6 gene. The vaccine formulation may further comprise a suitable carrier. Since TR6 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation instonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

The TR6 polypeptide of the present invention may be employed in a screening process for compounds which activate (agonists) or inhibit activation of (antagonists, or otherwise called inhibitors) the TR6 polypeptide of the present invention. Thus, polypeptides of the invention may also be used to assess identify agonist or antagonists from, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These agonists or antagonists may be natural substrates, ligands, receptors, etc., as the case may be, of the polypeptide of the present invention; or may be structural or functional mimetics of the polypeptide of the present invention. See Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

TR6 proteins are ubiquitous in the mammalian host and are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate TR6 polypeptide on the one hand and which can inhibit the function of TR6 polypeptide on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (eg inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (eg lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease.Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (eg inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (eg lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease.

Such screening procedures may involve producing appropriate cells which express the TR6 polypeptide of the present invention on the surface thereof. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells expressing the TR6 polypeptide (or cell membrane containing the expressed polypeptide) fused to the membrane and intracellular domains of any single transmembrane receptor, prefereably one with a known fuctional readout upon ligand binding (eg as tyrosine kinase domain) are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response.

The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the TR6 polypeptide is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor. Further, these assays may test whether the candidate compound results in a

signal generated by activation of the TR6 polypeptide, using detection systems appropriate to the cells bearing the TR6 polypeptide at their surfaces. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Standard methods for conducting such screening assays are well understood in the art.

Alternatively, TR6 may be expressed as a soluble protein, including versions which fuse all or part of TR6 with a convenient partner peptide for which detection reagents are available, eg TR6-IgG fusions, and used in a solid state or solution phase binding assay. For example, the soluble TR6 can be used to detect agonist or antagonist binding directly through changes that can be detected experimentally, eg surface plasmon resonance, nuclear magnetic resonance spectrometry, sedimentation, calorimetry. The soluble TR6 can be used to detect agonist or antagonist binding indirectly by looking for competition of the candidate agonist or antagonist with a ligand whose binding can be detected. Ligand detection methods include antibody recognition, modification of the ligand via radioactive labeling, chemical modification (eg biotinylation), fusion to an epitope tag. Methods include ELISA based assays, immunoprecipitation and scintillation proximity.

Assays similar to those described above using soluble or membrane bound TR6 may also be used to identify and purify the natural ligand(s) of TR6. These ligands may be agonists or antagonists of the receptor.

Examples of potential TR6 polypeptide antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates, receptors, etc., as the case may be, of the TR6 polypeptide, e.g., a fragment of the ligands, substrates, receptors, or small molecules which bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

The TR6 cDNA, protein and antibodies to the protein may also be used to configure assays for detecting the effect of added compounds on the production of TR6 mRNA and protein in cells. For example, an ELISA may be constructed for measuring secreted or cell associated levels of TR6 protein using monoclonal and polyclonal antibodies by standard methods known in the art, and this can be used to discover agents (i.e. antagonists or agonists) which may inhibit or enhance the production of TR6 from suitably manipulated cells or tissues.

Prophylactic and Therapeutic Methods

This invention provides methods of treating an abnormal conditions related to both an excess of and insufficient amounts of TR6 polypeptide activity.

If the activity of TR6 polypeptide is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of ligands to the TR6 polypeptide, or by inhibiting a second signal, and thereby alleviating the abnormal condition.

In another approach, soluble forms of TR6 polypeptides still capable of binding the ligand in competition with endogenous TR6 polypeptide may be administered. Typical embodiments of such competitors comprise fragments of the TR6 polypeptide.

In still another approach, expression of the gene encoding endogenous TR6 polypeptide can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al.*, *Nucleic Acids Res* (1979) 6:3073; Cooney *et al.*, *Science* (1988) 241:456; Dervan *et al.*, *Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of TR6 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates TR6 polypeptide, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of TR6 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited

therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996).

Formulation and Administration

Peptides, such as the soluble form of TR6 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 µg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a

polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Examples

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples illustrate, but do not limit the invention.

Example 1

An EST (EST#1760054; Project ID HSYAD88) with sequence similarity to the human TNF receptor was discovered in a commercial EST database. Analysis of the 1073 nucleotide sequence of the partial cDNA, indicated that it encoded an open reading frame for a novel member of the TNF receptor superfamily and was named TR6. The predicted partial protein is 307 amino acids long, with a hydrophobic membrane spanning region indicating that at least one form of TR6 is expressed as a membrane bound protein. Comparison of TR6 partial protein sequence, with other TNF receptor family proteins indicates that it has at least two of the cysteine-rich repeats characteristic of the extracellular domains of this family, and an intracellular death domain.

Example 2

Northern blot of TR6.

Various tissues and cell lines were screened for mRNA expression by Northern blot. RNA was prepared from cells and cell lines using Tri-Reagent (Molecular Research Center Inc., Cincinnati, OH), run in denaturing agarose gels (Sambrook et al., Molecular Cloning: a laboratory manual, 2nd Ed. Cold Spring Harbor Lab Press, NY (1989)) and transfered to Zeta-probe nylon membrane (Biorad, Hercules, CA.) via vacuum blotting in 25mM NaOh for 90 min. After neutralization for 5-10 minutes with 1M tris-HCl, pH 7.5 containing 3M NaCl, the blots were prehybridized with 50% formamide, 8% dextran sulfate, 6XSSPE, 0.1%SDS and 100mg/ml of sheared and denatured salmon sperm DNA for at least 30 min. At 42°C. cDNA probes were labeled with 32P-CTP by random priming (Statagene, La Jolla, CA), briefly denatured with 0.25M NaOH and added to the prehybridization solution. After a further incubation for at least 24h at 42°C, the blots were washed in high stringency conditions and exposed to X-ray film.

Very high expression of TR6 RNA was detected in aortic endothelial cells. High expression was also detected in monocytes. Low expression was detected in bone marrow and

CD4+ activated PBLs. Very low, but detectable levels of TR6 RNA was expressed in CD19+ PBLs, CD8+ PBLs (both activated and unstimulated), and unstimulated CD4+ PBLs.

In hematopoietic cell lines, low levels of TR6 RNA was expressed in HL60 (promyelocyte), KG1a (promyeloblast) and KG1 (myeloblast) cell lines. Very low but detectable levels of TR6 RNA was expressed in U937 (monoblast) and THP-1 (monocyte) cell lines.

The major RNA form is 3.5 kb in size.

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the polypetide of SEQ ID NO:2 or the corresponding fragment thereof; or a nucleotide sequence complementary to said nucleotide sequence.

2. The polynucleotide of claim 1 which is DNA or RNA.

3. The polynucleotide of claim 1 wherein said nucleotide sequence is at least 80% identical to that contained in SEQ ID NO:1.

4. The polynucleotide of claim 3 wherein said nucleotide sequence is contained in SEQ ID NO:1.

5. The polynucleotide of claim 1 wherein said encoding nucleotide sequence encodes the polypeptide of SEQ ID NO:2 or a fragment thereof.

6. A polynucleotide probe or primer comprising at least 15 contiguous nucleotides of the polynucleotide of claim 3.

7. A DNA or RNA molecule comprising an expression system wherein said expression system is capable of producing a TR6 polypeptide or a fragment thereof having at least 80% identity with a nucleotide sequence encoding the polypeptide of SEQ ID NO:2 or said fragment when said expression system is present in a compatible host cell.

8. A host cell comprising the expression system of claim 7.

9. A process for producing a TR6 polypeptide or fragment comprising culturing a host of claim 8 and under conditions sufficient for the production of said polypeptide or fragment.

10. The process of claim 9 wherein said polypeptide or fragment is expressed at the surface of said cell.

11. Cells produced by the process of claim 10.

12. The process of claim 9 which further includes recovering the polypeptide or fragment from the culture.

13. A process for producing a cell which produces a TR6 polypeptide or a fragment thereof comprising transforming or transfecting a host cell with the expression system of claim 7 such that the host cell, under appropriate culture conditions, produces a TR6 polypeptide or fragment.

14. A TR6 polypeptide or a fragment thereof comprising an amino acid sequence which is at least 80% identical to the amino acid sequence contained in SEQ ID NO:2.

15. The polypeptide of claim 14 which comprises the amino acid sequence of SEQ ID NO:2, or a fragment thereof.

16. A TR6 polypeptide or fragment prepared by the method of claim 12.

17. An antibody immunospecific for the TR6 polypeptide of claim 14.

18. A method for the treatment of a subject in need of enhanced TR6 polypeptide activity comprising:

(a) administering to the subject a therapeutically effective amount of an agonist to said polypeptide; and/or

(b) providing to the subject TR6 polynucleotide in a form so as to effect production of said polypeptide activity *in vivo*.

19. A method for the treatment of a subject having need to inhibit TR6 polypeptide activity comprising:

(a) administering to the subject a therapeutically effective amount of an antagonist to said polypeptide; and/or

(b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said polypeptide; and/or

(c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said polypeptide for its ligand, substrate, or receptor.

20. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of TR6 polypeptide in a subject comprising:

(a) determining the presence or absence of a mutation in the nucleotide sequence encoding said TR6 polypeptide in the genome of said subject; and/or

(b) analyzing for the presence or amount of the TR6 polypeptide expression in a sample derived from said subject.

21. A method for identifying compounds which bind to TR6 polypeptide comprising:

(a) contacting cells of claim 11 with a candidate compound; and

(b) assessing the ability of said candidate compound to bind to said cells.

22. The method of claim 21 which further includes determining whether the candidate compound effects a signal generated by activation of the TR6 polypeptide at the surface of the cell, wherein a candidate compound which effects production of said signal is identified as an agonist.

23. An agonist identified by the method of claim 22.

24. The method of claim 21 which further includes contacting said cell with a known agonist for said TR6 polypeptide; and

determining whether the signal generated by said agonist is diminished in the presence of said candidate compound, wherein a candidate compound which effects a diminution in said signal is identified as an antagonist for said TR6 polypeptide.

26. An antagonist identified by the method of claim 24.

ABSTRACT OF THE DISCLOSURE

TR6 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing TR6 polypeptides and polynucleotides in the design of protocols for the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (eg inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (eg lymphoproliferative disorders), atheroschlerosis, and Alzheimers disease.among others, and diagnostic assays for such conditions.

FIGURE 1. Nucleotide and Amino Acid sequence of TR6 (SEQ ID NOS: 1 and 2, respectively.)

```
          10                  30                  50
CCCACGCGTCCGATGACCTCCTTTTCTGCTTGCGCTGCACCAGGTGTGATTCAGGTGAAGTG
  HisAlaSerAspAspLeuLeuPheCysLeuArgCysThrArgCysAspSerGlyGluVal

          70                  90                  110
GAGCTAAGTCCCTGCACCACGACCAGAAACACAGTGTGTCAGTGCGAAGAAgGCACCTTC
GluLeuSerProCysThrThrThrArgAsnThrValCysGlnCysGluGluGlyThrPhe

          130                 150                 170
CGGGAAGAAGATTCTCCTGAGATGTGCCGGAAGTGCCGCACAGGGTGTCCCAgAGGGATG
ArgGluGluAspSerProGluMetCysArgLysCysArgThrGlyCysProArgGlyMet

          190                 210                 230
GTCAAGGTCGGTGATTGTACACCCTGGAGTGACATCGAATGTGTCCACAAAGAATCAGGC
ValLysValGlyAspCysThrProTrpSerAspIleGluCysValHisLysGluSerGly

          250                 270                 290
ATCATCATAgGAGTCACAGTTGCAGCCGTAGTCTTGATTGTGGCTGTGTTTGTTTGCaAg
IleIleIleGlyValThrValAlaAlaValValLeuIleValAlaValPheValCysLys

          310                 330                 350
TCTTTACTGTGGAAgAAAGTCCTTCCTTACCTGAAAGGCATCTGCTCAGGTGGTGGTGGG
SerLeuLeuTrpLysLysValLeuProTyrLeuLysGlyIleCysSerGlyGlyGlyGly

          370                 390                 410
GACCCTGAGCGTGTGGACAGAAGcTCACAACGACcTGGGGCTGAGGACAATGTCCTCAAT
AspProGluArgValAspArgSerSerGlnArgProGlyAlaGluAspAsnValLeuAsn
```

GH500008P


```
430                 450                 470
 .         .         .         .         .         .
GAGATCGTGAGTATCTTGCAGCCCACCCAGGTCCCTGAGCAGGAAATGGAAGTCCAGGAG
GluIleValSerIleLeuGlnProThrGlnValProGluGlnGluMetGluValGlnGlu

490                 510                 530
 .         .         .         .         .         .
CCAGCAGAGCCAACAGGTGTCAACATGTTGTCCCCCGGGGAGTCAGAGCATCTGCTGGAA
ProAlaGluProThrGlyValAsnMetLeuSerProGlyGluSerGluHisLeuLeuGlu

550                 570                 590
 .         .         .         .         .         .
CCGGCAGAAGCTGAAAGGTCTCAGAGGAGGAGGCTGCTGGTTCCAGCAAATGAAGGTGAT
ProAlaGluAlaGluArgSerGlnArgArgArgLeuLeuValProAlaAsnGluGlyAsp

610                 630                 650
 .         .         .         .         .         .
CCCACTGAGACTCTGAGACAGTGCTTCGATGACTTTGCAGACTTGGTGCCCTTTGACTCC
ProThrGluThrLeuArgGlnCysPheAspAspPheAlaAspLeuValProPheAspSer

670                 690                 710
 .         .         .         .         .         .
TGGGAACCCCTCATGAGGAAGTTGGGCCTCATGGACAATgAGATTgaGGTGGCTAAAGCT
TrpGluProLeuMetArgLysLeuGlyLeuMetAspAsnGluIleGluValAlaLysAla

730                 750                 770
 .         .         .         .         .         .
GAGGCAGCGGGCCACAGGGACACCTTGTACACGATGCTGATAAAGTGGGTCAACAAAACC
GluAlaAlaGlyHisArgAspThrLeuTyrThrMetLeuIleLysTrpValAsnLysThr

790                 810                 830
 .         .         .         .         .         .
GGGCGAGATGCCTCTGTCCACACCCTGCTGGATGCCTTGGAGACGCTGGGAGAGAGACTT
GlyArgAspAlaSerValHisThrLeuLeuAspAlaLeuGluThrLeuGlyGluArgLeu

850                 870                 890
 .         .         .         .         .         .
GCCAAGCAGAAGATTGAGGACCACTTGTTGAGCTCTGGAAAGTTCATGTATCTAGAAGGT
AlaLysGlnLysIleGluAspHisLeuLeuSerSerGlyLysPheMetTyrLeuGluGly
```

GH500008P

```
      910                 930                 950
       .                   .                   .
AATGCAGACTCTGCCATGTCCTAAGTGTGATTCTCTTCAGGAAGTCAGACCTTCCCTGGT
AsnAlaAspSerAlaMetSerEndValEnd

      970                 990                1010
       .                   .                   .
TTACCTTTTTTCTGGAAAAAGCCCAACTGGACTCCAGTCAGTAGGAAAGTGCCACAATTG

     1030                1050                1070
       .                   .                   .
TCACATGACCGGTACTGGAAGAAACTCTCCCATCCAACATCACCCAGTGGA
```